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THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS

III. THE RATE OF DIGESTION AND ABSORPTION AS DETERMINED BY EXPERIMENTS ON RATS

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IN CONNECTION with the work on the rate of absorption of cereals in human subjects (1) it seemed desirable to make similar experiments upon another animal. Mature rats were used for this purpose and the method employed was similar to the one used by Cori (2) in his study of the absorption of sugars. Applied to the study of solid foods, instead of solutions, this method has certain limitations, but the results were nevertheless of some interest.

The method was briefly as follows: To each of five or six rats which had fasted 48 to 72 hours there was fed an exactly weighted amount of cereal cooked uniformly for fifteen minutes. The amount consumed was usually about five grams, equivalent to about a gram of the dry cereal. The exact time when the meal was consumed was noted in each case, and at varying intervals thereafter one of the group was sacrificed and the cereal remaining in the stomach and in the small intestine was recovered, ligatures being placed about the cardiac, pyloric, and ileocolic sphincters before the alimentary tract was removed. As a measure of the amount of cereal present a determination of starch seemed most trustworthy, and for a basis of calculation the same determination was made upon a portion of the original cooked cereal.

The determination of starch was made as follows: To the cooked cereal, or gastro-intestinal contents, about 250 cc. of water and 5 cc. of concentrated HCl were added and this mixture was boiled under a reflux condenser for 3 hours. After cooling, neutralizing, and making up to a definite volume, an aliquot was analyzed for reducing sugar by the method

of Bertrand with one modification: the precipitated cuprous oxide was separated and washed by centrifugation instead of by filtration. In the hands of two workers the procedure went smoothly and rapidly and the possibility of reoxidation was reduced to a minimum. When the duplicate analyses failed to agree, additional determinations were made.

Since the animals of a series were not of identical weight, (usually 150 to 200 grams) the figures for cereal (starch) fed and recovered were all put on the basis of 100 gram weight of rat for the purpose of comparison. The amounts of cereal recovered from the stomach and small intestine were each divided by the amount fed and the quotients, expressed in terms of percentage, thus gave a measure of the amount of cereal still present in the stomach and remaining unabsorbed in the intestine. More than 100 animals were used in the entire series.

Before the results are discussed in detail two limitations of the method must be mentioned. The first concerns variations in the motility of the alimentary tract which are more pronounced with respect to solids than with respect to liquids. Furthermore, different animals required variable lengths of time to consume the meal. Some took 15 minutes; others required thirty. Insofar as it was possible to do so, these differences were equalized by using the slowly eating animals for the three and four hour examinations; animals requiring a longer time were omitted from the series. These and other biological variations could not be ruled out except by sufficient observations to treat statistically.

A second limitation in the precision of the method concerns the validity of the figures for reducing sugar as an index of the amount of starch and cereal involved. This applies less to the original cereal sample than to the partially digested gastro-intestinal contents. Glucose is presumably absorbed as rapidly as it is produced by digestion, but the method makes no distinction between starch and its partial decomposition products, the lower dextrins and maltose. The amount of material available was too small to permit a separation of these forms of reducing sugar. Despite these sources of error, starch was a better basis for this work than any of the other cereal constituents. Even in a 48-hour fasted rat the coecum contains considerable amounts of potential reducing material; hence the coecum was omitted from consideration.

The cereals studied were Wheat Endosperm (3), "Whole Wheat" and Pre-cooked Oats. The rates of their passage from the stomach and of the absorption of their starch content are shown in the accompanying charts, in which the amounts of cereal present in stomach and intestine are plotted against time. In the construction of these smoothed curves all the values were used except a few isolated ones shown at the extreme right

in some of the charts. Through delayed motility a residuum of starch was still present in these animals and values could not be put into the averages.

The curves in Charts 1, 2, and 3 represent the gradually decreasing amounts of the cereal foods recovered from stomach and small intestine and from both together, *i.e.*, the rate of passage from the stomach and the rate of absorption in the intestine.

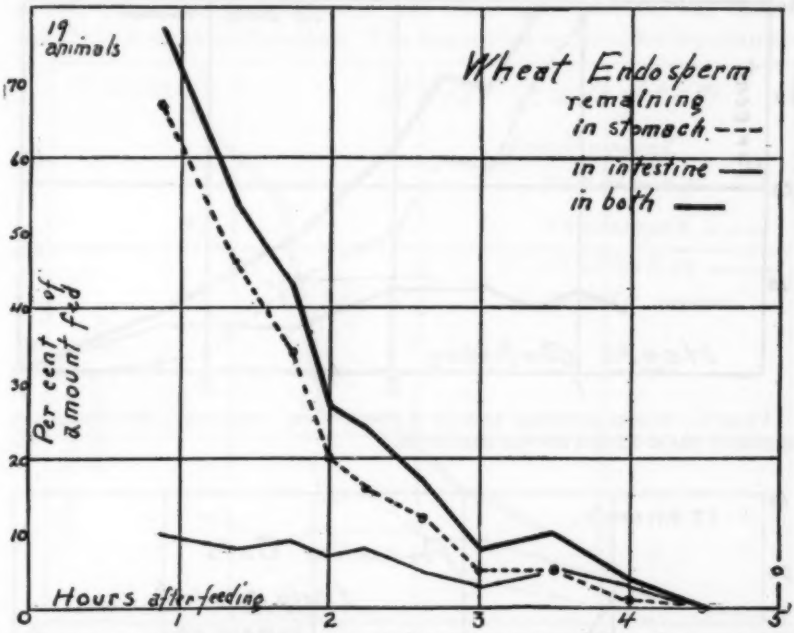

CHART 1.—Average percentage amounts of wheat endosperm remaining in the stomach and in the intestine of rats at different intervals after feeding.

A comparison chart is not necessary to demonstrate that the rate of absorption of Wheat Endosperm was slower than that of "Whole Wheat" during the first two hours. Thereafter they were practically parallel. The relations in the first two hours are, on first consideration, the reverse of what might be expected on the basis of the usual conception that the larger the food particles, the slower they are to leave the stomach. However even a naked-eye examination of the two cereals showed that in Wheat Endosperm the grains were of fairly uniform size while the bran-containing cereal had some large and some very small particles. Further, and as a matter of cooking experience, the presence of bran flakes prevents cereal from coagulating into a solid gummy mass when cooked, as is the case with a bland cereal. Conditions would thus be favorable in the other

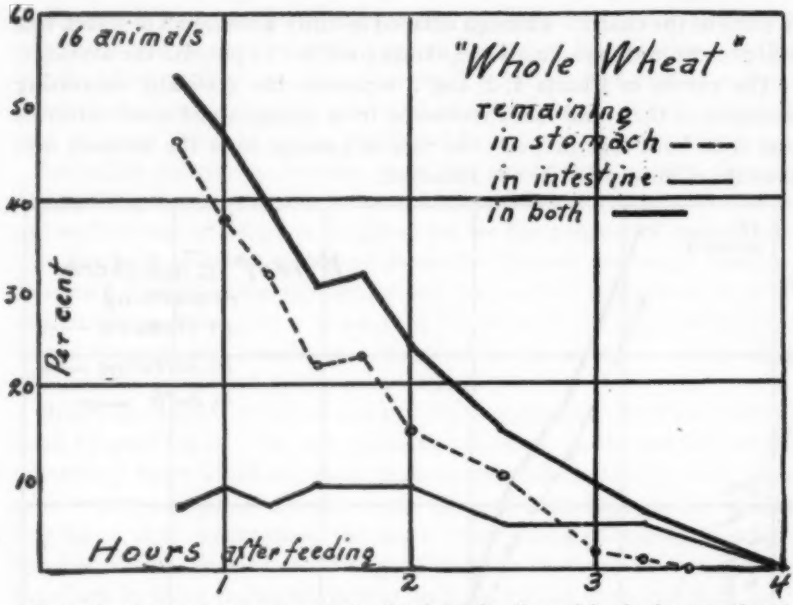

CHART 2.—Average percentage amounts of "whole wheat" remaining in the stomach and intestine of rats at different intervals after feeding.

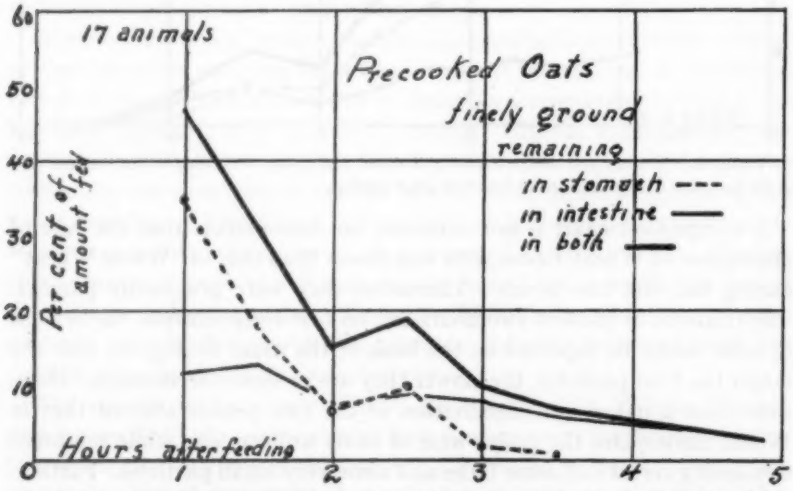

CHART 3.—Average percentage amounts of precooked oats remaining in the stomach and intestine of rats at different intervals after feeding.

for the early departure of the smaller starch particles upon the measure of which the whole experiment depended.

The difficulties encountered in the examination of Precooked Oats tended to confirm this interpretation. In order to assure uniform samples for the parallel analyses it was felt necessary to grind this cereal to a fine flour, and as the chart reveals, the stomach emptying time of this cereal, despite its higher protein and crude fiber content, was shorter than that of either of the others. The longer time required for its complete

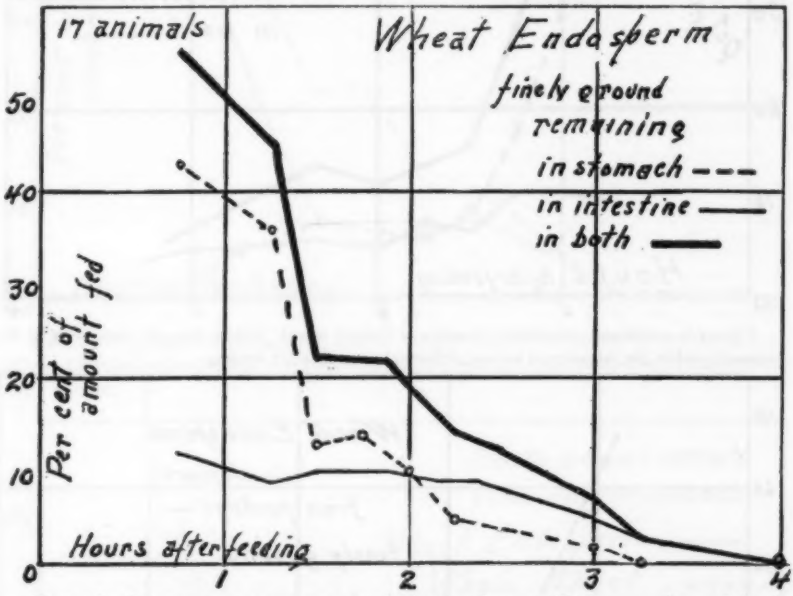

CHART 4.—Average percentage amounts of wheat endosperm (finely ground) remaining in the stomach and in the intestine of rats at different intervals after feeding.

disappearance from the small intestine is in keeping with the usual opinion expressed in the phrase that it "stays by one longer."

It became necessary, therefore, to try the two wheat preparations similarly ground to a fine flour. The data from these tests are given in Charts 4 and 5. That they leave the stomach more rapidly in this form than when used directly from the package is shown in Charts 6 and 7. Finally on Chart 8 are the curves for the emptying time of the stomach for all three breakfast cereals in finely ground form. Within the limits of the method, the three curves are in good agreement and we have, apparently, further proof of the generally accepted fact just mentioned that the more finely divided a food the more rapidly it leaves the stomach.

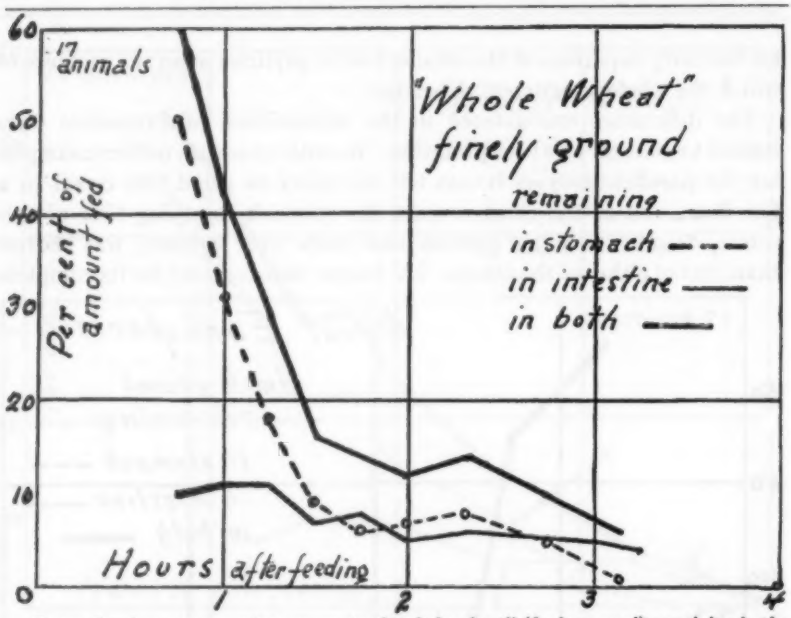

CHART 5.—Average percentage amounts of "whole wheat" (finely ground) remaining in the stomach and in the intestine of rats at different intervals after feeding.

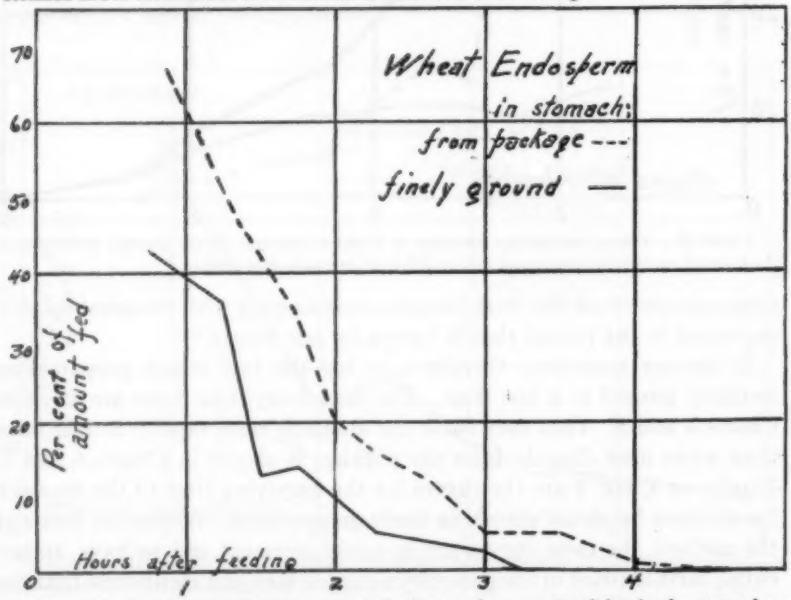

CHART 6.—Average percentage amounts of wheat endosperm remaining in the stomach at different intervals after feeding, when fed as obtained from the package and when fed after being finely ground.

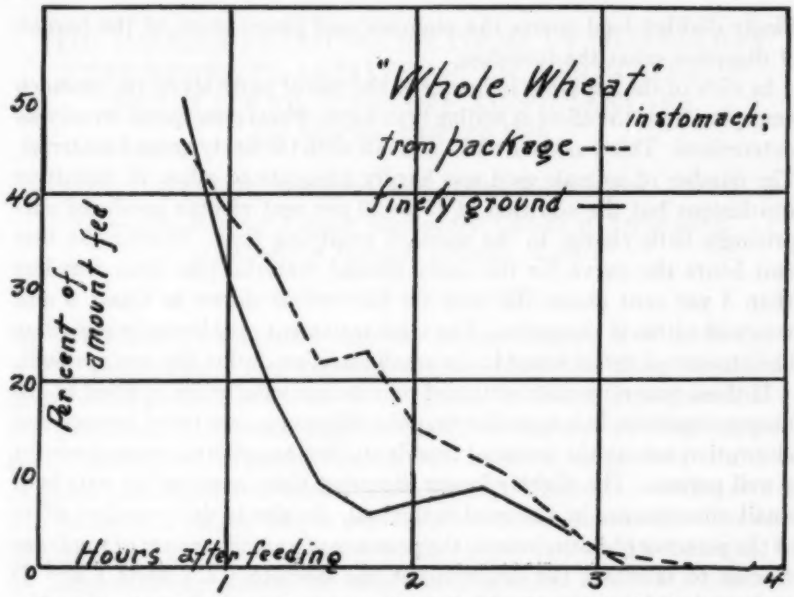

CHART 7.—Average percentage amounts of "whole wheat" remaining in the stomach at different intervals after feeding, when fed as obtained from the package and when fed after being finely ground.

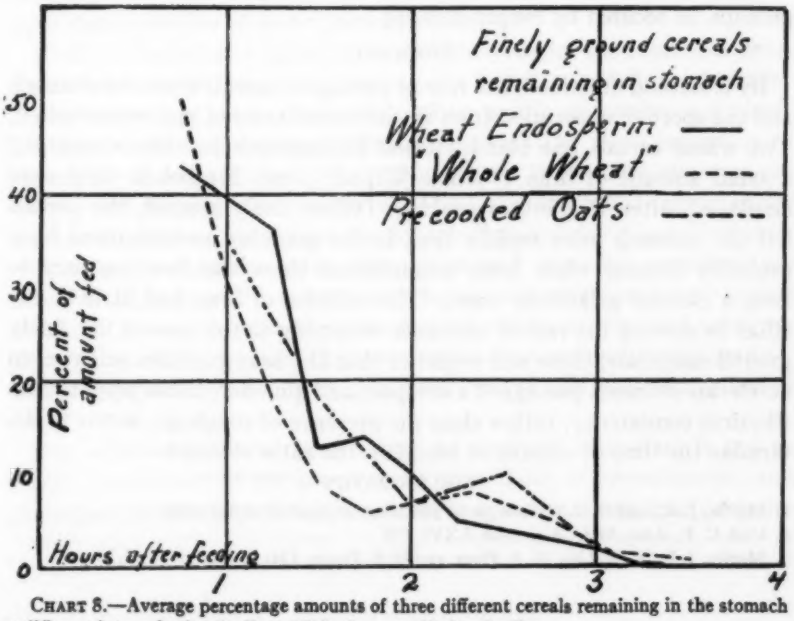

CHART 8.—Average percentage amounts of three different cereals remaining in the stomach at different intervals after feeding. All finely ground before feeding.

Finely divided food spares the stomach and places more of the burden of digestion upon the intestine.

In view of the apparent influence of the size of particles on the stomach emptying time, the effect of adding bran to the wheat endosperm cereal was determined. This was done on 12 animals with the finely ground material. The number of animals used was hardly adequate to allow of definitive conclusions but the addition of 15 to 20 per cent of bran produced surprisingly little change in the stomach emptying time. During the first two hours the curve for the finely ground material plus bran was less than 5 per cent above the zone for the cereals shown in Chart 8 and was well within it thereafter. The same statement may be made regarding the amount of cereal found in the small intestine during the same periods.

If these general results obtained on rats are valid when applied to the human organism, it is apparent that the differences in rates of passage and absorption among the forms of cereals studied are of little importance for a well person. The slightly longer digestion time required by oats is of small consequence in a normal individual. So also is the retarding effect of the presence of bran; indeed, the presence of a small amount of roughage seemed to facilitate the emptying of the stomach (cf. Charts 1 and 2) perhaps by aiding the breaking up of a compact gelatinous mass. The increased rate of stomach emptying produced by fine grinding of the product (Charts 6 and 7) may be credited to the same influence and could perhaps be secured by longer cooking.

Summary

By a method described, the rate of passage of cereals from the stomach and the speed of absorption from the intestinal tract of rats were studied. Two wheat cereals, one bland, (Wheat Endosperm) the other containing a small amount of bran ("Whole Wheat"), and Precooked Oats were employed, after 15 minutes cooking. When finely ground, the cereals left the stomach more rapidly than in the granular manufactured form probably because when finely comminuted there was less tendency to form a gummy gelatinous mass. The addition of bran had little if any effect in slowing the rate of stomach emptying in the case of the finely ground materials; there was evidence that the bran particles might even accelerate stomach passage of a compact and glue-like cereal preparation. Physical consistency, rather than the presence of roughage, seems to determine the time of sojourn of cereal in the rat's stomach.

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THE PAIRED-FEEDING METHOD IN NUTRITION EXPERIMENTS AND ITS APPLICATION TO THE PROBLEM OF CYSTINE DEFICIENCIES IN FOOD PROTEINS

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METHODS of research may be divided roughly into methods of measurement and methods for the control of those conditions under which measurements are to be taken. In animal experimentation, the methods of measurement employed include methods of collecting gaseous, liquid, and solid excreta, calorimetric methods, methods of chemical analysis, methods of determining physical properties, such as temperature, and methods of taking weight and dimensional measurements. The importance of accuracy in this type of method is self-evident. For example, in biochemical investigations, the value of experimental results is recognized as dependent primarily on the accuracy of the chemical methods used. Experiments employing analytical methods of little, or even of questionable, accuracy may be practically obliterated from the literature by the mere demonstration of such a state of affairs.

In the biological sciences in particular, methods for the control of those conditions under which measurements are to be taken are fully as important as the methods of measurement themselves, and in many phases of nutrition these methods assume a paramount position. The rapid expansion of the science of nutrition in the last twenty years is largely a direct consequence of the introduction of a method of controlling the nutrient intake of experimental animals by means of synthetic diets composed largely of individually purified constituents. The use of such diets has permitted the effective study of each known dietary factor with reference to any animal function. It has also led to the demonstration of the existence of unknown dietary factors and to their characterization by systematic investigation. The biological analysis of foods is an outgrowth of this factorial method of study.

But unfortunately the effectiveness of methods of control is not so readily ascertained as the effectiveness of methods of measurement, and consequently their imperfections are not so apparent nor so clearly demon-

strable. Frequently the evaluation of a method of experimental control is more a matter of logic than of laboratory investigation. It is perhaps for this reason that faulty methods of control persist in common usage more often and for a longer time than faulty chemical methods. The employment of improperly controlled experimental procedures means not only ineffectual experimentation, yielding results defying interpretation, but also the acceptance as facts of experimental interpretations that are not securely established. Even though an experimental interpretation of this character ultimately proves to be correct, it must be reassessed before it can possess any claim to finality.

It is a relatively easy matter to control an experiment with reference to the peculiarities of individual behavior by employing a number of animals properly selected, or by letting each animal be its own control in a succession of properly selected periods of experimental treatment and observation. The equalization of environmental conditions of temperature, humidity, light, shelter, confinement, etc., among the different animals or groups of animals to be compared is generally recognized as an important requisite to a clear solution of the problem under investigation. In nutrition experiments the composition of the experimental diets is often the main item of concern, and frequently the greatest care is taken in the compounding of rations to the end that between any two rations only one constituent shall be variable. But the control of the amount of food consumed has always been the most neglected and the most baffling problem in nutrition investigations, and the failure to control the food intake of experimental animals has been the direct cause of much ineffective experimental work.

In 1912, F. G. Hopkins published a report of feeding experiments on rats illustrating the importance of the food consumption of animals in the interpretation of such data (1). This paper possesses the historical importance of foreshadowing the discovery of the vitamins and constitutes Hopkins' claim to recognition in this connection. In the experiments reported, young rats were offered synthetic diets made up of isolated proteins, fats, carbohydrates, and a salt mixture. Other rats were offered the same ration, but with the addition of a small amount of milk. The rats receiving no milk soon failed to grow, while the rats receiving the milk addendum, amounting to 4 per cent or less of the dry matter in the ration, grew normally and continuously. The food intakes of the rats were not controlled, but their importance in the interpretation of the experiments was fully recognized. Almost 10 pages of this report, or almost exactly one third of the text, are concerned with the question of the relation of the growth secured to the amount of food consumed. Only after he was

able to assure himself, necessarily by indirect means, that the difference in the growth observed between the milk rats and the non-milk rats could not have been due solely to the difference in the amounts of food consumed, was Hopkins prepared to conclude that the milk addendum exerted a direct effect upon the growth processes.

This classical experiment testifies to the fact that the proper control of nutrition experiments should extend beyond the control of the composition of the diet to the control of its consumption by experimental animals. The painstaking care of Hopkins in making the most of a confusing situation, apparently not anticipated in the planning of the experiment, established his conclusion with what appears to be a high degree of probability, but undoubtedly the interpretation would have been more direct and more convincing if the food intake of his milk rats had been restricted to that of his non-milk rats. If one ration is superior to another in the support of an animal function such as growth, its superiority should be evident when the intake of both rations by comparable animals is the same, either absolutely or in proportion to some determinant of food requirements, such as body weight, or a mathematical function of body weight. In fact, such an equality of food intake is essential to a clear-cut demonstration of a difference in nutritive value.

The Paired-Feeding Method

The problem of the control of food intake is thus an important one in animal experimentation; it is also a difficult one to solve. We propose to consider a plan of food control that seems hopeful of success, and that actually, in a number of laboratories, has given results capable of only one interpretation, surely an unmistakable earmark of effective experimentation.

The first experiment in the literature, in so far as we have been able to discover, in which this method of food control was used, is a cooperative experiment planned by the late Dr. Armsby in 1917 and sponsored by the National Research Council. The purpose of these investigations, which involved eight state agricultural experiment stations, was to compare the effect upon the growth of calves, as measured by body weight, body dimensions, and the rate of nitrogen retention, of two rations containing different percentages of essentially the same protein mixture. The point of immediate interest is the method by which this comparison was to be effected. To quote from the plan of the experiment:

"The plan contemplates the comparison of the animals by pairs, one animal of each pair receiving the high-protein and one the low-protein ration. The two animals of each pair should be

of substantially the same breed (not necessarily full-bloods) and as nearly as possible of the same age and weight at the beginning of the experiment.

"It is desired that as many pairs be used as is consistent with the keeping of individual records. Lot records are of far less value. The more nearly alike the several pairs are the better, but uniformity in this respect is not so essential as between the two animals of each pair." (2).

Omitting the details of the management of the calves during the growing period, it is necessary to point out only two items of control as bearing upon the question under discussion. The first item is that "if the proportion of roughage in the rations proves to be too great, the quantity of oat straw may be reduced *for both animals of a pair* by the same amount per 1000 pounds live weight." The second item refers to the adjustment of the energy supply of the animals "in order, on the one hand, to prevent fattening or, on the other, to maintain good condition. This may be effected by varying the amount of starch used, but *the energy supply of both animals of a pair must be kept the same per 1000 pounds live weight.*" The italics are Armsby's.

Although the results of these experiments were not sufficiently significant nor concordant to afford any definite conclusion concerning the relative growth-promoting value of the high-protein and low-protein rations, the fault did not lie in the plan of the investigations, but in the indifferent success with which the plan was carried out. Either intentionally or inadvertently the net energy intakes of the paired calves were not equalized as directed, so that the observed differences in growth cannot be interpreted with reference to the difference in protein intake only.

The determination of the nutritive deficiencies of rations of which experimental animals, either immediately or eventually, will not consume enough for the maintenance of weight, has proved to be a difficult problem. The results obtained cannot be interpreted on the basis of their face value. In laboratory investigations there is a marked tendency to assume that the refusal of rations by experimental animals is proof *ipso facto* that they are inadequate in one or more nutritive constituents. But this is not a critical interpretation. In experiments of this character, the paired-feeding method seems to offer marked advantages, and in actual experimental work had proved its value.

Essentially this plan of food control has been employed with highly significant results in studies of the physiological effects of rations deficient in those water-soluble vitamins until recently unified under the term "vitamin B." The particular need of control of food consumption in such investigations resides in the fact that the appetite of animals subsisting upon these deficient rations ultimately is seriously impaired, even to the extent of total refusal of food. The question arises, therefore, to what

extent are the physiological effects produced due to a specific vitamin hunger, and to what extent are they due to a generalized undernutrition or even to inanition. Strange as it may seem, this point has not been considered in the large majority of all investigational work of this character on vitamins.

One of the physiological effects ascribed to a lack of the so called "vitamin B" is a severely depressed basal heat production. In 1922, and again in 1924, Gulick (3) investigated this particular relation by the paired-feeding method. He found that the basal metabolism of rats was indeed severely depressed to a level less than 70 per cent of the normal by subsistence upon a "vitamin-B-free" diet. But their food intakes were also severely depressed. If for each test rat, a litter mate control was fed upon the same diet plus a small amount of "vitamin-B" supplement (dried yeast), and in the same amounts, restricted in accordance with the diminishing food consumption of the test rat, a severe depression in basal metabolism also resulted, similar in magnitude to that observed in the rat receiving no "vitamin B." Thus, the depression in metabolism in rats receiving a ration deficient in "vitamin B" was shown conclusively to be unrelated to the vitamin deficiency, but to be the result of a generalized undernutrition.

The results of Gulick were confirmed four years later by Drummond and Marrian (4), who showed that the nutritive failure in rats following a deficiency of "vitamin B" is virtually identical with that resulting from starvation, and that the starved rats exhibited nerve lesions indistinguishable from those found in "vitamin-B" deficient rats.

In the following year Kon and Drummond (5) applied the paired-feeding method to a similar study on pigeons. The distinction between the effects of inanition and those of vitamin deficiency was made possible by the control of the food intake of both of a pair of birds of equal weight to the amount voluntarily eaten by the one on the deficient diet. The experiment, which involved a number of such pairs, proved that most of the symptoms attributed to a dietetic deficiency of "vitamin B," except adrenal hypertrophy and acute nervous symptoms, were present equally in the control bird and its pair mate, even the degenerative changes in the peripheral nerves that have been considered a specific result of a lack of the antineuritic vitamin. In commenting upon this remarkable outcome of their experiment, Kon and Drummond say, "No existing theory is considered as giving a satisfactory explanation of the role of vitamin B in the normal organism, or of the true nature of the conditions induced by its deficiency."

The paired-feeding method has recently been used by Rose, Stucky, and Cowgill (6) in studies of the relationship between the depressed gastric motility observed in dogs subsisting upon "vitamin-B-free" food and the deficiency in vitamins. Again the effects of undernutrition (as well as of hunger) in the etiology of this condition were established.

When rations are offered to experimental animals *ad libitum*, many factors, known and unknown, contribute to the determination of the amounts of feed actually consumed. Among the known factors may be mentioned the flavor, texture, nutritive completeness and available energy content of the ration. If two rations are being compared with reference to their inherent nutritive values, a difference in the intake of each by the experimental animals is to be expected. But by permitting a difference in food intake to develop between animals whose growth is to be compared, the interpretation of the difference in growth in terms of the make-up of the rations is difficult and may even become impossible. The argument is well illustrated by experiments concerned with the value of common salt as a supplement to cereal rations. The condimental value of salt is well known, so that the mere demonstration that animals grow better when given cereal rations plus salt than when given cereal rations alone may mean simply that a more liberal consumption of the rations has thus been induced. However, at the Nutrition Laboratory of the University of Illinois (7) a ration based mainly upon corn, when supplemented with salt, induced 50 per cent faster gains in weight of both rats and chickens than the salt-less ration, even when the food consumption of the paired animals was kept the same. The deficiency of corn in either sodium, or chlorine, or both, was thus clearly established.

A similar paired-feeding investigation with growing pigs, concerned with the supplemental value of ferrous sulfate (copperas) added to rations both high and low in iron, was reported in 1927 by Carroll and Mitchell (8).

If the food intakes of the pair mates have been strictly equalized throughout the period of experimental feeding, the paired-feeding method is capable of a high degree of accuracy, such as is needed in the detection of small differences in the nutritive balance of rations. For 4, 5, or 6 pairs the outcome of the test should be identical in all pairs, if a significant difference in nutritive value exists between the two rations tested. The results of the method, however, are peculiarly susceptible to simple statistical treatment, since there can be no suspicion that the selection of the animals interferes in the slightest with the randomness of the differences between pair mates in growth, blood composition, bone composition, or other measurement taken. In fact, the more successful the selection of pair mates has been in the direction of equality of initial capacity to grow

and develop on the basal ration, the more clear-cut will be the statistical interpretation. In contrast with this is the ordinary group-feeding method. Here the more effective the selection of animals in equalizing the growth capacities of the groups to be compared, the more the randomness of the gains obtained within groups is disturbed, and the less applicable become the ordinary statistical methods in the interpretation of the results secured. And yet, without the aid of statistical methods the results of the ordinary group-feeding experiment frequently cannot be precisely interpreted.

Its Application to the Problem of Cystine Deficiencies

The paired-feeding method is admirably adapted to the problem of the determination of the amino acid deficiencies of proteins and mixtures of proteins, upon which their supplementary relations depend. Some work has been done upon the cystine deficiencies of a number of food products and it is this work upon which a detailed report will be made here.

A preliminary survey of a number of foods was first made, using only 3 or 4 pairs of rats in each case. These foods were skim milk powder, white bread, potato, navy beans, beef muscle, and canned green garden peas. The bread was a bakery product containing milk, and was dried only. The potatoes were baked in an oven, peeled, sliced, and dried at a low temperature in a current of warm air. The navy beans were soaked in distilled water, boiled thoroughly, and dried without waste. The beef muscle was obtained as round steak. The lean was freed of all visible fat, ground, dried, and extracted with ether. The peas were a local brand of canned garden peas. They were boiled for about 15 minutes and dried without waste.

After drying and grinding, all foods were analyzed for nitrogen and made up into rations containing approximately 8 per cent of protein ($N \times 6.25$), furnished entirely by an individual food. For each food a second ration was made up containing 0.24 per cent of cystine and approximately 7.76 per cent of protein. The composition of these rations is given in Table I.

Four pairs of rats were started upon each food, one rat in each pair to receive the unsupplemented ration and the other rat the ration containing added cystine. In each pair the rats were of the same sex, frequently of the same litter, and in all cases of the same weight within a few grams. They were kept in individual cages and fed weighed amounts of their respective rations, the food intake being controlled so that the two rats of a pair received as nearly as possible the same amount of food per week, determined by the rat consuming the least. After the first 5 weeks of feeding each rat received an additional daily vitamin supply as cod liver oil and dried yeast or yeast extract. All rats were weighed weekly.

TABLE I

THE COMPOSITION OF THE RATIONS

	Nitrogen	Protein	All values in percentage by weight											
			A	B	C	D	E	F	G	H	I	J	K	L
Milk powder	5.735	35.84	22.32	21.65	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Bread	2.116	13.23	.....	.....	60.49	58.68	.....	.....	.....	.....	.....	.....	.....	.....
Potato	1.683	10.52	.....	.....	.....	.....	76.05	73.77	.....	.....	.....	.....	.....	.....
Beans, navy	3.946	24.66	.....	.....	.....	.....	.....	.....	32.44	31.47	.....	.....	.....	.....
Beef, dried	14.782	92.39	.....	.....	.....	.....	.....	.....	.....	.....	8.66	8.40	.....	.....
Pea, garden	3.286	20.54	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	38.95	37.78
Cystine	11.51	....	.....	0.24	.....	0.24	.....	0.24	.....	0.24	.....	0.24	.....	0.24
Salts, Osborne and Mendel	.....	....	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Butter fat	.....	....	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	.....	....	10.00	10.00	10.00	10.00	4.95	6.99	10.00	10.00	10.00	10.00	10.00	10.00
Cellu Flour	.....	....	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Sodium chloride	.....	....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Starch	.....	....	48.68	49.11	10.51	12.08	.....	.....	38.56	39.29	62.34	62.36	32.05	32.98
Totals	.....	....	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nitrogen	.....	....	1.239	1.264	1.278	1.322	1.273	1.267	1.354	1.263	1.279	1.254	1.333	1.269
Protein	.....	....	7.741	7.901	7.988	8.263	7.956	7.919	8.463	7.894	7.994	7.838	8.442	7.931

One pair of rats on the potato ration was withdrawn from the experiment very soon after it began because of respiratory disease. Also one of the rats on the pea ration died after 4 weeks of feeding.

The results of this preliminary experiment have been condensed and summarized in Table II. The items listed in this table are self-explanatory except the last, described as "comparison of weekly gains." The corresponding figures give for each rat the number of weeks for which its gain surpassed that of its pair mate. In the case of equal gains, 0.5 is credited to each pair mate.

In the case of beef muscle and white bread, the cystine supplement was evidently ineffective in promoting more rapid gains. In the former case in 3 of the 4 pairs the cystine rat gained the more, but of the 48 weekly comparisons for all pairs the control rat was ahead in 22.5 cases and the cystine rat in 25.5 cases, a close approximation to equality. In the white bread experiment, the control rat gained the more in each pair, but this may well have been a chance result with such a small number of pairs. If an event may occur with equal probability in either of 2 ways, then in a random selection of 4 trials, the frequencies of its occurrence in one way 0, 1, 2, 3, and 4 times is given by the expansion of the binomial distribution

$$(\tfrac{1}{2}+\tfrac{1}{2})^4.$$

Its occurrence 4 times in either way would result from chance once in 16 trials. Hence, with 4 pairs of rats, a consistent outcome favoring either the control or the test rats would result from chance only, twice in 16 trials. A detrimental effect of cystine in this case seems improbable in view of the results on other pairs, so that this particular outcome may well be considered fortuitous. Of the 59 weekly comparisons among these 4 pairs of rats, 34 favored the control rat and 25 the cystine rat.

Among the three pairs of rats on the potato ration, the total gains of the cystine rats in all cases exceeded those of their pair mates, while of the 48 comparisons of weekly gains, the cystine rat was favored in 33 and the control rat in only 15. The former result cannot be handled statistically in a satisfactory manner because only 3 pairs were used, but the latter result may be analyzed further on the assumption that, if the cystine supplement is without value, the chances are even that in any one week on equal intakes of food the gain in weight from either ration will exceed that from the other. Hence, in the present case, if the nitrogen compounds of potato were not improved in growth-promoting value by the cystine supplement, the ideal outcome of 48 comparisons of the weekly gains of paired rats would be 24 favoring the control rat and 24 favoring the cystine rat. The question at issue is whether the deviation from this

TABLE II

THE CYSTINE DEFICIENCIES OF FOOD PROTEINS AS DETERMINED BY THE PAIRED-FEEDING METHOD

(All weights in grams)

Food investigated	Data	Pair 1		Pair 2		Pair 3		Pair 4	
		Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Peas, cooked	Initial weight	71	65	130	132	41	40	38	36
	Final weight	79	92	130	178	51	59	67	94
	Gain	8	27	0	46	10	19	29	58
	Total food	288	281	485	488	109	109	273	281
	Days on food	77	77	77	77	28	28	77	77
	Comparison of wk. gains	1.5	9.5	0.5	10.5	0	4	1	10
Beef muscle, fat-free	Initial weight	51	53	45	43	56	56	68	64
	Final weight	146	169	159	158	163	160	197	217
	Gain	95	116	114	115	107	104	129	153
	Total food	542	533	508	507	562	555	648	646
	Days on food	84	84	84	84	84	84	84	84
	Comparison of wk. gains	4.5	7.5	5.5	6.5	7.5	4.5	5	7
Navy beans, cooked	Initial weight	62	60	57	60	63	62	63	61
	Final weight	80	85	80	94	74	83	80	91
	Gain	18	25	23	34	11	21	17	30
	Total food	268	274	302	299	250	261	301	301
	Days on food	77	77	77	77	77	77	77	77
	Comparison of wk. gains	4.5	6.5	4	7	5	6	3	8

TABLE II (*Continued*)

THE CYSTINE DEFICIENCIES OF FOOD PROTEINS AS DETERMINED BY THE PAIRED-FEEDING METHOD

TABLE II (*Continued*)

THE CYSTINE DEFICIENCIES OF FOOD PROTEINS AS DETERMINED BY THE PAIRED-FEEDING METHOD

(All weights in grams)

Food investigated	Data	Pair 1		Pair 2		Pair 3		Pair 4	
		Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Potato, baked	Initial weight	52	52	46	46	62	62		
	Final weight	76	88	119	139	115	148		
	Gain	24	36	73	93	53	86		
	Total food	504	498	666	666	701	700		
	Days on food	112	112	112	112	112	112		
	Comparison of wk. gains	6	10	6	10	3	13		
White bread	Initial weight	51	50	55	56	64	64	55	55
	Final weight	98	95	79	73	104	103	96	84
	Gain	47	45	24	17	40	39	41	29
	Total food	477	476	304	301	472	472	426	423
	Days on food	112	112	77	77	112	112	112	112
	Comparison of wk. gains	9.5	6.5	6	5	9	7	9.5	6.5
Skim milk	Initial weight	52	51	45	46	50	50	49	48
	Final weight	187	205	180	214	194	234	167	163
	Gain	135	154	135	168	144	184	118	115
	Total food	815	810	797	793	1000	1006	746	753
	Days on food	99	99	99	99	112	112	99	99
	Comparison of wk. gains	5.5	8.5	3	11	3.5	12.5	8.5	5.5

ideal, obtained in this particular experiment, *i.e.*, $33-24=9$, could reasonably result from the operation of chance, which would have determined the deviation if the cystine supplement were without nutritive effect. This again is a proposition involving the assessment of probabilities by means of the binomial distribution. The standard deviation of the frequency distribution of the outcome of 48 events, each of which may result with equal probability in either of two ways, is given by the expression $\sqrt{0.5\times 0.5\times 48}$,[1] which is equal to 3.46. The deviation of 9 from the expected outcome is 2.6 times this standard deviation. It would occur by chance only once in 107 trials,[2] so that this method of analysis indicates clearly that cystine actually supplemented the potato ration and induced more rapid gains than would have resulted otherwise on the same intake of food.

Of the four pairs of rats on the navy bean ration, the cystine rat gained distinctly more rapidly in all pairs, though the rate of gain was extremely small in all cases. This result would have been obtained by chance once in 16 trials and, therefore, in itself it proves nothing. Of the 44 comparisons of weekly gains in all 4 pairs, 27.5 favored the cystine rat, a deviation of 5.5 from the expectation, if chance alone had determined the outcome. The standard deviation in this case, computed as explained above, is 3.32; the deviation of 5.5 is only 1.66 times this value, an outcome that may have resulted from chance once in 10 trials. This method also fails to establish a sound basis for the belief that cystine has effectively supplemented the proteins of navy beans. However, neither of these methods of analysis takes full advantage of the data, since they consider only the sign of the differences in the gains of pair mates, not their magnitudes. In view of the inconclusive nature of the results obtained thus far, the analysis should be continued further on the latter basis. The average of the 44 weekly differences in the gains of paired rats is 0.932 gram in favor of the cystine animal. The standard deviation of these differences is 2.94 grams, and the probable error of the average difference, 0.30 gram. Since the average difference is more than 3 times its probable error, it may be considered significant. It appears probable that cystine has effectively supplemented the proteins of navy beans, but that, under the conditions of this experiment, the effect was too small to be conclusively demonstrated with only 4 pairs of rats.

The results with skim milk were rendered somewhat inconclusive by the fact that for one pair of rats the control animal gained 3 grams more

[1] See R. A. Fisher: Statistical Methods for Research Workers. Edinburgh and London, 1928. Chapter III.

[2] According to Davenport's Table of Values of the Normal Probability Integral.

than the cystine animal in 99 days of feeding. Of the 58 comparisons of weekly gains, however, the cystine rat was favored in 37.5, a deviation of 8.5 from the mid-value of 29. The standard deviation is this case ($\sqrt{0.5 \times 0.5 \times 58}$) equals 3.81. The deviation is 2.23 times its standard deviation, and would be obtained by chance only once in 39 trials. Proceeding further with these differences, the average difference in weekly gain between paired rats with its probable error is $+1.40 \pm .43$, the sign indicating that the cystine rats showed the larger average gain. In spite of the erratic behavior of one pair of rats, the analysis may be taken to mean that, with a high degree of probability, cystine effectively supplemented milk proteins in their growth-promoting value.

The 4 pairs of rats on the ration containing, as its source of protein, canned garden peas, gave unmistakable evidence that the dietary protein was deficient in cystine. In spite of the very slow gains made, the effect of the added cystine was clear. In all 4 pairs of rats, the total gain of the cystine rat exceeded considerably the total gain of its control. Of the 37 weekly comparisons of gains between pair mates, 34 favored the cystine rat and only 3 the control, an outcome extremely improbable by the operation of chance factors only.

It seemed desirable to repeat the tests upon some of these foods with a larger number of pairs of animals, both as a further test of the method, and as a more satisfactory test of the foods. The fact that one pair of rats on the skim milk diets gave evidence contrary to that of the other 3 pairs, the latter evidence confirming the conclusion of Sherman and Merrill (9) that milk proteins are deficient in cystine, made it seem desirable to repeat the test on this food. Although the data on garden peas were remarkably consistent and clean cut, the conclusion that this variety of *Pisum sativum* possesses proteins deficient in cystine, while, according to Finks, Jones, and Johns (10), the field pea belonging to the same species either in the uncooked or cooked condition, does not, would seem to need further investigation.

Accordingly the experiment with skim milk was repeated with 9 pairs of rats, and that with garden peas with 6 pairs of rats. Another brand of canned garden peas was used in the latter test. The results of the tests are summarized in Tables III and IV.

In the milk test, the rat receiving the cystine supplement gained more than its pair mate in 10 weeks of feeding in each of the 9 pairs. This result would have been obtained by chance only once in 512 trials. Of the 90 comparisons of the weekly gains of paired rats, 73.5 favored the rat receiving the cystine supplement. The ideal result, if chance alone operated,

TABLE III

THE VALUE OF CYSTINE AS A SUPPLEMENT TO THE PROTEINS OF WHOLE MILK

(All weights in grams)

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Pair 9	
	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine	Con-trol	Cys-tine
Initial weight	57	56	55	52	51	51	47	45	40	40	40	41	41	37	32	34	34	34
Final weight	163	177	153	152	146	164	141	165	152	189	148	173	135	171	128	164	137	151
Gain	106	121	98	100	95	113	94	120	112	149	108	132	94	134	96	130	103	117
Total food	717	712	599	606	662	657	613	611	619	622	594	594	595	595	575	578	544	541
Days fed	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
Comparison of wk. gains	4	6	3.5	6.5	3	7	1.5	8.5	0	10	1.5	8.5	1	9	0	10	2	8

TABLE IV

THE VALUE OF CYSTINE AS A SUPPLEMENT TO THE PROTEINS OF COOKED GARDEN PEAS

(All weights in grams)

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	117	115	103	100	107	108	52	50	50	47	46	45
Final weight	150	168	117	132	141	166	90	101	87	98	83	97
Gain	33	53	14	32	34	58	38	51	37	51	37	52
Total food	755	745	550	565	711	721	398	398	378	369	395	387
Days fed	113	113	113	113	113	113	106	106	106	106	106	106
Comparison of wk. gains	5.5	10.5	5	11	3	13	4	11	5	10	3	12

would be 45, and the significance of the deviation of actual and theoretical results, *i.e.*, $73.5-45=28.5$, must be assessed. The standard deviation in this case is $\sqrt{0.5\times0.5\times90}=4.74$. The deviation is almost exactly 6 times the standard deviation and could thus hardly have resulted from chance. Both methods of analysis, therefore, agree in showing that milk proteins are undoubtedly deficient in cystine, and would be effectively supplemented by cystine-rich proteins.

In the experiment on garden peas, the percentage of pea protein in the rations was initially made approximately 8 per cent, but after 3 weeks of feeding this percentage was increased to 12 per cent because of the slow growth that was being obtained. Neither ration was well eaten and the growth throughout the experiment was very slow. Nevertheless, in spite of these adverse conditions, the paired-feeding method was capable of showing unmistakably that the proteins of cooked green garden peas are deficient in cystine. All six pairs placed the cystine rat ahead of its pair mate in total gain during approximately 15 or 16 weeks of feeding. Such a result would have been obtained by chance only once in 64 trials. Of the 93 comparisons of weekly gains between pair mates, 67.5 favored the cystine rat, a deviation from the chance expectation (46.5) of 21. This is 4.3 times the standard deviation (4.82), and could not be considered within reason to be the resultant of chance factors only.

"Student's" method (11) for analyzing the significance of small groups of differences between test and control observations may also be applied to paired-feeding experiments such as this. The average differences between the total gains of the 6 pair mates is 17.3 grams, and the standard deviation of differences is 3.83 grams. The ratio of the mean difference to the standard deviation, the z of "Student," is 4.5. With $z=4.5$ and $n=6$, according to the table of "Student," the odds are well over 2000 to 1 that the cystine supplement was responsible for the rapid gains of the rats consuming the cystine ration.

In connection with the experiment on garden peas, the digestibility of the pea protein, with and without the cystine supplement, was determined from 7 day collections of feces, once on the 8 per cent protein ration and again on the 12 per cent protein ration. The coefficients of digestibility are summarized in Table V.

Cystine does not seem to have affected the digestibility of the proteins of peas, according to these figures. On the 8 per cent ratio, the average coefficient for the control rats was 79.5 and for the cystine rats 77.8. On the 12 per cent ration, the averages were 80.0 and 80.3, respectively.

It has been demonstrated by Mitchell and Carman (12) that the gains

in weight of growing rats may not be fair measures of the nutritive values of the rations upon which they have been subsisting, because of the fact that gains may vary widely in chemical composition, particularly as regards fat. Does this observation vitiate the significance of the comparisons of gains secured in paired-feeding experiments? In an attempt to

TABLE V

THE COEFFICIENTS OF DIGESTIBILITY OF NITROGEN OF THE GARDEN PEA RATIONS WITH AND WITHOUT THE CYSTINE SUPPLEMENT

	Protein Content of Ration			Protein Content of Ration	
	8 pct.	12 pct.		8 pct.	12 pct.
Pair 1			Pair 4		
Control	83.8	85.1	Control	79.5	83.4
Cystine	74.9	80.3	Cystine	70.2	79.4
Pair 2			Pair 5		
Control	74.2	78.4	Control	77.6	75.2
Cystine	90.2	83.9	Cystine	79.4	76.5
Pair 3			Pair 6		
Control	78.1	82.8	Control	83.7	75.4
Cystine	79.8	83.5	Cystine	72.2	78.3

answer this question, the rats in the milk experiment described above were analyzed after removal of the contents of the alimentary canal. The results of this analysis are summarized in Table VI.

With two exceptions the percentage of protein in the cystine rats exceeded that of their pair mates, and for all pairs the protein content in grams of the cystine rat was greater than that of the controls. It is interesting to note that with Pair 2, in which the control rat and the cystine rat gained approximately the same and attained to almost identical final weights, the carcass of the control rat contained 22.43 per cent of ether-soluble matter, while the cystine rat contained only 14.18 per cent. Also, the weight of protein in the latter animal was appreciably greater than in the former. Evidently on the same kind of food except for the cystine content, and on the same amount of food, the control rat fattened to a much greater extent than the cystine rat. In Pair 7, the reverse seems to have occurred. Among the other pairs of rats the fat content of pair mates did not vary widely. It may be significant that the greatest divergencies among pair mates in the percentage of fat in the carcass, *i.e.*, in

Pairs 2, 3, and 7, occurred in pairs made up from rats of different litters. The rats in Pairs 1, 5, 6, and 8 were litter mates.

It may be concluded that these analytical data substantiate, rather than vitiate, the live weight comparisons of paired rats, and that in the case of Pair 2 they explain satisfactorily an approximate equality of gain in live

TABLE VI

CHEMICAL COMPOSITION OF RATS RAISED ON THE MILK RATION WITH AND WITHOUT CYSTINE

	Empty weight gms.	Percentage Composition				Protein content gms.
		Dry matter	Crude protein	Ether extract	Ash	
Pair 1						
Control	160	41.39	18.81	18.65	4.00	30.1
Cystine	174	41.94	18.19	19.03	3.81	31.7
Pair 2						
Control	151	43.51	16.81	22.43	3.96	25.4
Cystine	150	36.98	18.31	14.18	4.44	27.5
Pair 3						
Control	142	38.68	18.56	15.39	4.28	26.4
Cystine	157	37.93	20.50	13.42	4.19	32.2
Pair 4						
Control	141	41.97	17.25	19.04	4.24	24.3
Cystine	165	42.22	18.19	19.57	3.87	30.0
Pair 5						
Control	152	38.71	18.13	16.40	3.72	27.6
Cystine	180	40.68	18.88	16.37	3.83	34.0
Pair 6						
Control	144	36.75	17.94	13.73	3.84	25.8
Cystine	169	37.55	19.19	13.78	3.99	32.4
Pair 7						
Control	133	39.58	18.25	17.55	4.14	24.3
Cystine	168	45.07	17.25	22.94	3.73	29.0
Pair 8						
Control	126	38.58	18.88	15.09	4.24	23.8
Cystine	162	40.05	19.00	16.41	3.99	30.8
Pair 9						
Control	137	39.28	18.13	16.16	3.81	24.8
Cystine	148	40.33	18.88	16.70	4.23	27.9

weight that is at variance with the belief that cystine has effectively supplemented milk proteins.

Summary and Conclusions

The paired-feeding method has been used effectively in a number of investigations in which the effect of a variable food intake must be eliminated to permit of a clear interpretation of the results with reference to the one deliberately imposed variable. Its results may be readily analyzed by simple statistical methods.

The method is particularly well adapted to a study of the amino acid deficiencies of proteins and of the mixtures of proteins and non-protein nitrogenous compounds occurring in natural foods. In this experiment it has been used in the determination of the existence of cystine deficiencies among a number of food products. The results appear to justify the following conclusions:

The proteins of lean beef and of white bread are not deficient in cystine since their growth-promoting value for rats is not enhanced by supplementing with cystine.

The proteins of navy beans, potatoes, milk, and garden peas are deficient in cystine in supplying the growth requirements of the rat. A repetition of the experiments on milk and garden peas confirmed strongly the results of the preliminary experiments on only 4 pairs of rats.

From the experiments on peas, in particular, it appears that the paired-feeding method is capable of giving clear-cut results under the adverse condition of inadequate food consumption by the experimental animals.

The addition of cystine to the garden pea ration did not modify the completeness of digestion of the nitrogenous compounds contained in it.

Further evidence is presented to show that the fat content of the gains of rats made on the same amounts of rations of nearly identical make-up may differ widely.

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Note.—Since this article was written our attention has been called to the fact that the paired-feeding method has been used by W. E. Anderson and A. H. Smith (J. Biol. Chem. 1924, LXI, 181–191) in a study of the effect of acute scurvy on subsequent nutrition and growth of guinea pigs. "It was convincingly demonstrated by the carefully controlled methods employed that the loss of body weight observed in the present series of scorbutic guinea pigs at the height of scurvy cannot be accounted for by the inanition factor alone. Growth in the postscorbutic stage is either parallel to or greater than that of the normal guinea pig and is accompanied by a definite increase of food intake."

THE EFFECT OF INSULIN UPON THE BODY WEIGHT OF THE RABBIT

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Introduction

SEVERAL diabetic patients of the Potter Metabolic Clinic, dietitians, and others interested in the treatment of diabetes, have felt that the use of insulin brings about an abnormal gain in weight. This idea, as far as we can gather, has not in any case been based upon a statistical survey. It would, indeed, be difficult to conceive of carrying out such a study on the diabetic patient. The severe diabetic, before taking insulin, is undernourished and underweight, and a gain in weight following insulin therapy is therefore to be expected. The mild diabetic, on the other hand, is often overweight. Joslin's studies (1) have shown rather conclusively that the incidence of diabetes is higher in the obese. When the severe diabetic on insulin treatment becomes obese he may be merely returning to his prediabetic weight. In the foreign literature (2, 3, 4, 5, 6) there are reports in which undernourished non-diabetic patients gained weight under the influence of insulin. The insulin is regarded as stimulating the appetite, so that a higher calorie intake of food is induced.

In this paper two pertinent questions concerned with the effect of insulin upon body weight are considered. 1. Does the administration of insulin to the normal animal, without the simultaneous augmentation of the caloric intake of food, bring about an increase in weight? 2. Does the administration of insulin to the normal animal stimulate the appetite, so that more food is eaten? Rabbits were used as experimental animals. In one series of experiments rabbits receiving doses of insulin and controls not receiving insulin were placed upon weighed diets. In the other series the rabbits receiving insulin and the controls were permitted to eat as much food as they desired, a record of the food intake and body weight being kept.

Experimental

Series 1. Six rabbits, 4 males and 2 females, of the same litter, were started at 3 months of age on a weighed diet, consisting of 87.5 gms. alfalfa and 26 gms. of barley totaling approximately 250 calories daily. Three of

these rabbits, Nos. 3, 5, and 6, two males and one female, were given daily doses of 6 units of insulin subcutaneously. This was increased at the end of six weeks to 8 units. Rabbits Nos. 1, 2, and 4 served as controls. The intake of barley remained the same throughout the experiment; the alfalfa was increased after 5 weeks to 157 gms. per day, making the daily caloric intake 340, and decreased two weeks later to 120 gms., giving 280 calories per day.

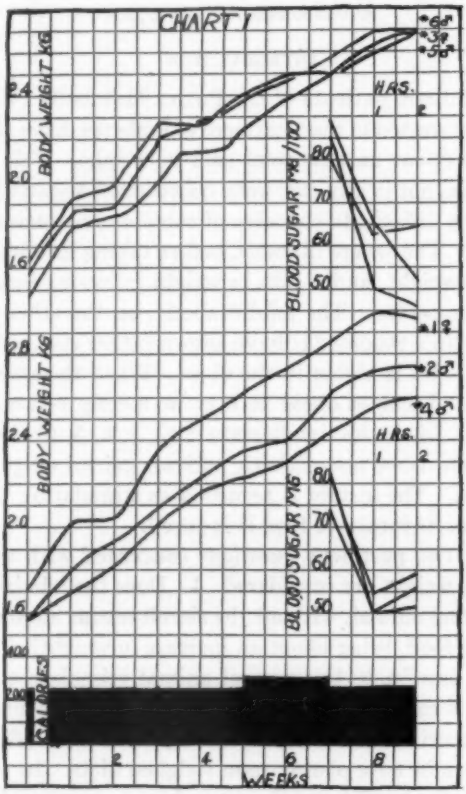

CHART 1.—Rabbits on restricted, weighed diet of barley and alfalfa.

Upper curves—growth and blood sugar curves of rabbits receiving daily subcutaneous doses of insulin.

Lower curves—growth and blood sugar curves of control rabbits.

The rabbits were weighed weekly. No differences in weight were observed between the rabbits receiving insulin and those not receiving it. The experiment was terminated at the end of ten weeks. At this time blood sugar

curves following insulin were determined for all of the rabbits on the experiment. This was done to be sure that a sufficiently large dose of insulin had been given to produce a distinct hypoglycemia.

Series II. In the second series the rabbits were given as much alfalfa as they would eat, and a weighed amount of barley each day. Six rabbits, four males and two females of the same litter, having an average weight of 1.7 kg., were started on the diet when 6 weeks old. Rabbits Nos. 7, 8,

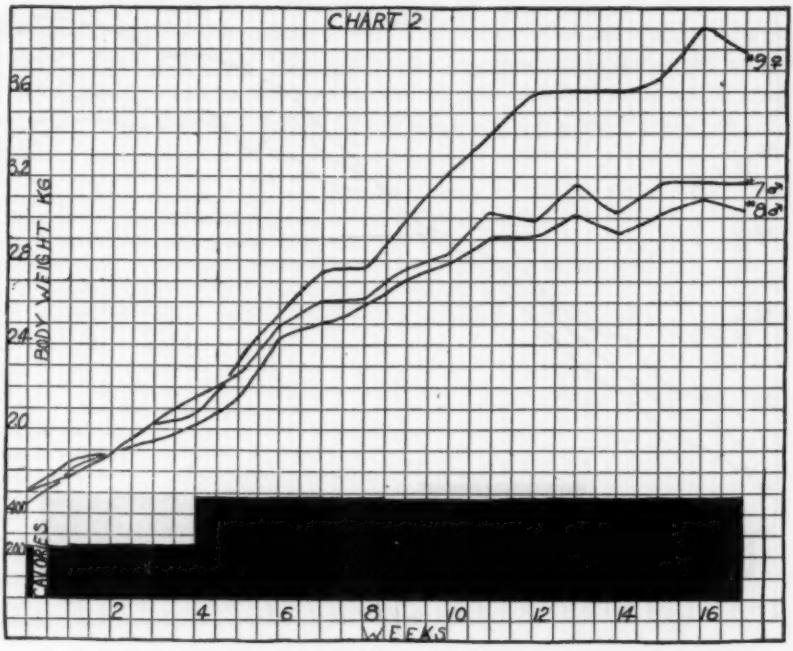

CHART 2.—Rabbits on an unrestricted diet of alfalfa and a weighed amount of barley.

Growth curves and caloric intake of rabbits in second series which received daily subcutaneous doses of insulin.

and 9, two males and a female, were given daily doses of insulin, beginning with 6 units and increasing by two units each two weeks until fourteen units were being given daily during the last month of the experiment. All were fed alfafa *ad lib.*, a record being kept of the daily amount consumed during the first two months and a quantity in excess of this given during the remainder of the experiment. Twenty six grams of barley were given daily the first month and 52 grams thereafter.

The average daily intake of alfalfa for the rabbits taking insulin was 144.5 grams, and for the controls 152.8. An analysis of the barley and alfafa was made in order to determine the caloric intake of the rabbits.

	Barley	Alfalfa
Protein	9.4%	16.7%
Fat	2.3%	1.8%
Carbohydrate	78.7%	17.7%
Calories per 100 gm.	373.0	154.0

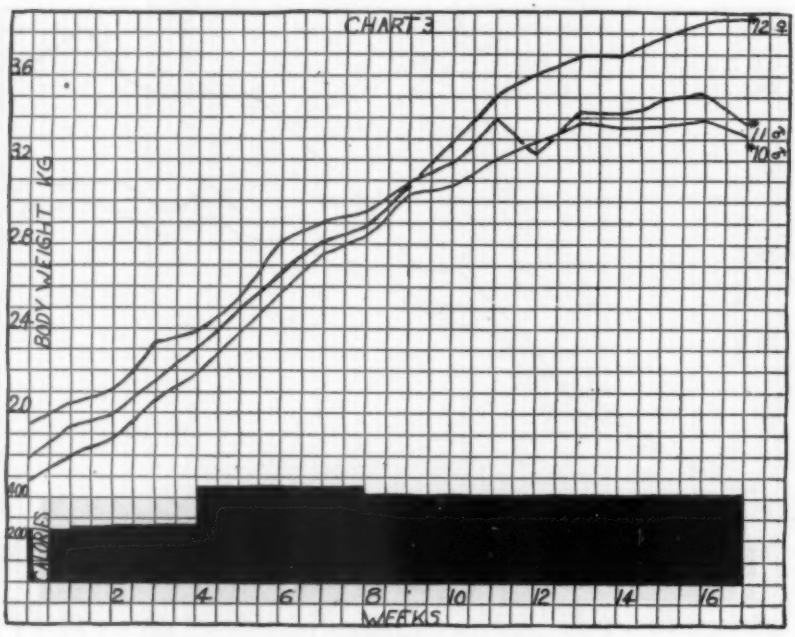

CHART 3.—Rabbits on an unrestricted diet of alfalfa and a weighed amount of barley. Growth curves and caloric intake of control rabbits in second series.

This would make their daily caloric intake as follows:

	Alfalfa	Barley	Total
Insulin rabbits, 1st mo.	223	97	320
Remainder of experiment	223	194	417
Controls, 1st mo.	235	97	332
Remainder of experiment	235	194	429

As can be seen from the growth curves, the increase in weight was practically the same for the two groups of rabbits.

Conclusion

Feeding experiments started upon young rabbits and carried through to maturity show that insulin does not bring about increased gain in weight either when the calorie intake is restricted, or when the animals are permitted to eat as much as they desire. Since the animals were not diabetic, no definite conclusion can be made in regard to the diabetic patient. It would be impossible to settle the question upon depancreatized animals because of the digestive disorders following the removal of the pancreas. It would also be impossible to establish the pre-diabetic weight of a patient. One is, therefore, tempted to draw a parallelism between the normal rabbit and the human diabetic, and to say that insulin does not cause obesity in the diabetic, but merely brings him back to his pre-diabetic weight.

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VITAMIN STUDIES XVII. OSSIFYING POTENCY OF RAW AND EVAPORATED MILKS*

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THE investigations described in this paper are a continuation of our work on the vitamin content of raw and evaporated milk. Previous papers have dealt with the amount of vitamin A and of the B complex found in the milk of the Pennsylvania State College herd, and in the evaporated milk made therefrom. The present data represent an attempt to evaluate the ossifying potency of whole and treated milk.

In regard to the antirachitic potency of milk, published data exhibit conflicting results. Steenbock, Hart, Hoppert and Black (1), using their yellow corn ration (No. 2965), found that 12 cc. (daily) of cows' milk were the minimum requirement for the healing of rickets in rats. Luce (2) reported that, 2 to 5 cc. of milk from pasture-fed cows would prevent rickets in rats fed on the McCollum ration (No. 3143), but that 15 to 20 cc. of milk from cows kept in the dark were ineffective. Hess and Weinstock (3) stated that, in an experiment of the preventive type, 20 to 25 cc. of milk prevented rickets. Outhouse, Macy and Brekke (4), using the Osborne and Mendel rachitogenic ration, and a curative technique, found that "30 cc. of cows' milk fed daily for 7 days induced marked healing of rachitic lesions in rats." Differences in the environment and feeding of the cows as well as in rations, in animals, and in the experimental technique, make explanation of these conflicting results difficult.

In the present experiments the milk came from the college certified herd which is kept under conditions carefully standardized as to rations, exercise, and light. As described in our previous papers (5, 6), the evaporated milks were made from the same sample as the raw milk fed, and under conditions approximating those used in commercial practice. In this way the evaporated milks could be tested before and after sterilization. From June until January five kinds of milk, raw (RM), vacuum evaporated (VE), vacuum-evaporated-sterilized (VES), air-evaporated (AE), and air-evap-

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orated-sterilized (AES), were fed. Before feeding, the evaporated milks were diluted to the concentration of the raw milks in order to compare them with equal volumes of the raw certified milk from which they were made.

The animals were chosen carefully from the "Penn State strain" of pied rats reared in this laboratory on a diet the constituents of which have not been varied for several years (7). Each animal was placed in an individual cage and given the Steenbock yellow corn ration (No. 2965) (8) for 21 days, with the prescribed volume of milk in separate doses daily. Lighting conditions were uniform throughout, and no sunshine, even through glass, was allowed to fall on the cages. Equal numbers of male and female rats, 21 days of age and weighing between 36 and 42 grams, were evenly distributed throughout the experiment. At this age the amount of ash in the femurs of our experimental animals averaged about 42 per cent. At the end of the 21-day feeding period, the rats were killed with ether and examined carefully for beading of ribs, enlargement of joints, and softness of bones. The right humerus was preserved for the line test of McCollum and co-workers (9), and the femurs were removed for ashing according to our previous technique (7). The bones were dried at constant temperature in the electric oven for 24 hours, extracted with 95 per cent alcohol, and again with ether for the same length of time, dried, and ashed in a muffle furnace at about 600° C. On the basis of the dry extracted bone, the percentage of ash and the ratio of ash to organic matter (A/R) were calculated.

The data are summarized in Table I. The bone-ash recorded represents group averages and when distinct groups were fed the same quantity of milk at different seasons, the averages are given separately and, later, together. The raw milk was fed in 5, 10, 12, 15, and 20 cc. portions. The 5 and 10 cc. doses produced a bone-ash of 35 and 39 per cent, respectively, and both were below the original 42 per cent level of the bone-ash in the femurs at the time when the animals were placed on the experimental diet. The addition of 12 cc. of raw milk raised the bone-ash to 43.4 per cent, which is higher than the original level and yet not so high as the ash (53 per cent) produced in animals of the same age which have been fed on the stock ration for the same length of time. Since 5 and 10 cc. of raw milk (daily) did not increase the bone-ash and, since the ingestion of 20 cc. was very difficult for such small animals during the first part of the feeding period, 10, 12 and 15 cc. quantities were chosen as the levels at which the evaporated milks were to be fed.

The milks were fed from June, 1926, to January, 1927, and during July,

1927, the experiment was continued as before with the exception that equal amounts of milk-ash were fed to ascertain the contribution made by the minerals alone. The seasonal variations in ossification were not marked in animals receiving 10 cc. except in the group receiving raw milk, which may have been due to the feeding of small amounts of green feed to the college herd in June, the month in which one of the two determinations on raw milk was made. On the 12 cc. level seasonal variations were evident, in all the experimental groups in which two seasons were represented, and since in each case July was one of the months represented in the group, the ration given to the cows may account for the difference in the ossifying potency of the milk. This coincides with the experience of Luce (2) who found greater "antiricketic value" in milk from the same cow when she was pasture fed than when eating a dry ration.

The experimental milks were fed at the 10 cc. level and the percentages of bone-ash obtained were as follows: RM 38.3, VE 33.4, VES 27.8, AE 29.5, AES 25.5. When 12 cc. were fed, the percentages of bone-ash were RM 43.4, VE 39.3, VES 38.1, AE 35.0, AES 34.8. At the 15 cc. level, the percentages of bone ash produced were RM and VE 44.8, VES 44.1, AE 42.5, and AES 40.3. The differences between the milks are less marked at this level, possibly because, with this quantity, both the minerals and the calcifying potency of the milk are increased to the point where deposition is equal or superior to loss of bone salts.

According to Daniels and Loughlin (10), some of the differences between the evaporated and raw milks may be due to the mechanical removal of a portion of the mineral salts from the milk by the evaporation process. Evaporated milks made from the same original sample by the vacuum method and by the air-evaporation method, when fed in the same amounts produce a distinct and different lowering of the bone-ash. A difference in the method of evaporation might produce a difference in the mineral content and, therefore, a difference in the amount of ash in the bones of animals to which the two types of milk were fed, but when especial care was taken to prevent the loss of minerals by the use of glass containers, sterilization again produced a lowering in the bone-ash. Therefore, some of the variations in the bone-ash may have been due to loss of antirachitic potency as well as to direct loss of minerals in the evaporation process.

The bone-ash of the animals fed 20 cc. of raw milk averaged 48.8 per cent. This is nearly a normal value and agrees with the statement of Krauss (11), using 0.8 gm. of butter fat, that 23 cc. milk are required for normal bone formation in rats fed a rachitic ration. Our data on 20 cc. of raw milk and on evaporated milks are not in agreement with Daniels

and Jordan (12), but, owing to lack of detailed data in their preliminary report and the apparent differences in technique, we are unable to explain the discrepancies at the present writing. Furthermore, the amount of antirachitic factor in our milks must have been much less than that observed by Daniels and Jordan, since they irradiated their milk samples with ultra violet light. It has been our experience that it is often necessary to feed a vitamin at the "threshold" level in order to note, with certainty, its susceptibility to heat treatment.

To determine the effect of the minerals alone on the rate of ossification, samples of raw, vacuum-evaporated, and vacuum-evaporated-sterilized milks were ashed. The ash was dissolved in dilute hydrochloric acid and made up with distilled water to the original volume of the milk from which the ash was prepared. One group of rats were fed 12 cc. aliquots, while their litter mates received the same volume of the original samples of milk. Unfortunately, at this time, air-evaporated milk samples were not available, since the evaporator had been removed from the dairy plant on the assumption that no more milk would be needed for this experiment.

The results of the feeding of the milk ash are shown in Table I. The bone of the rats which received ash from raw milk averaged 36.3 per cent, 9 per cent lower than their litter mates which were fed the same volume of raw milk. The separate groups of animals which received the two evaporated milks yielded approximately the same bone-ash, 33.8 and 33.7 per cent. The difference between the results given by the vacuum-evaporated milk and the same milk sterilized is practically negligible. The three groups which received milk ash produced a bone-ash which does not differ materially from that obtained with the air-evaporated milks, sterilized and unsterilized. These are the samples on which the greatest amount of injury was noted.

According to Chick and Roscoe (13), "The larger variations in bone composition seem to be confined to the water, fat, and mineral constituents. It seems probable that the value of the ratio, mineral ash: organic residue, might after all be the most trustworthy guide to the efficiency of the process of calcification." Therefore, the A/R ratios were calculated and are given in the table. Compared with the percentage of bone-ash they show the same general trend, *i.e.*, as the amount of bone-ash grows larger, the A/R ratio increases.

The line tests showed that the wide and irregular bands of cartilage had little or no deposition of calcium salts in the provisional zone of calcification at their base. As the cartilage bands became narrower and straighter, the line at the bases became heavier, showing that calcium salts had been

TABLE I

Milk	cc.	Month	No. of animals	Bone Ash per cent	A/R	Mean		Line test cartilage band
						Ash	A/R	
RM	5	June	8	35.2	.542	35.2	.542	
	10	June	7	40.0	.667	38.3	.622	Wide—fairly
		Dec.	8	36.9	.584			regular
	12	Dec.	12	41.7	.716	43.4	.775	Wider than normal
		July	10	45.5	.846			bone—regular
(*RM Ash*)	12	July	10	36.3	.572	36.3	.572	Wide—irregular
	15	Aug.	10	44.9	.817	44.8	.812	Narrower than 12 cc.
		Oct.	4	44.6	.805			RM—regular
	20	Aug.	8	48.8	.953	48.8	.953	Nearly like normal bone—regular
VE	10	Sept.	9	34.2	.521	33.4	.502	Wide—irregular
		Dec.	6	32.2	.475			
	12	Jan.	10	36.9	.586	39.3	.651	Wider than 12 cc.
		July	10	41.7	.716			RM—regular
(*VE Ash*)	12	July	10	33.8	.513	33.8	.513	Wide—irregular
	15	Oct.	9	44.8	.812	44.8	.812	Wider than 15 cc. RM—regular
VES	10	Sept.	10	27.7	.384	27.8	.388	Very wide—
		Dec.	6	27.9	.395			irregular
	12	Jan.	10	35.4	.548	38.1	.618	Wider than 12 cc.
		July	10	40.7	.688			VE—irregular
(*VES Ash*)	12	July	10	33.7	.511	33.7	.511	Very wide— irregular
	15	Oct.	10	44.1	.778	44.1	.778	Wider than 15 cc. VE —fairly regular
AE								
	10	Sept.	10	29.3	.414	29.5	.420	Very wide—
		Dec.	6	29.8	.432			irregular
	12	Jan.	10	35.0	.540	35.0	.540	Wider than 12 cc. RM—irregular
	15	Jan.	10	42.5	.743	42.5	.743	Wider than 15 cc. RM—regular
	10	Sept.	9	25.4	.340	25.5	.344	Very wide—
AES		Dec.	5	25.8	.351			irregular
	12	Jan.	10	34.8	.534	34.8	.534	Wider than 12 cc. AE—irregular
	15	Jan.	10	40.3	.673	40.3	.673	Wider than 15 cc. AE—fairly regular
Breeding ration						52.9	1.12	Narrow band— very regular

laid down. This band became narrower and more regular as the A/R ratio and the percentage of the bone-ash increased.

Summary

Prophylactic experiments are described in which milk was added to a rachitic diet. Evaporated milks made by vacuum and aeration methods were fed at different levels in direct comparison with the raw milk from which they were made. A slight seasonal variation is shown in both the raw and evaporated milks.

The ossifying potency of the treated milks is less than that of the raw. Vacuum evaporation, aeration, and sterilization tended to decrease the ossifying potency of raw milk.

Ash, equivalent to 12 cc. of raw, vacuum-evaporated, and vacuum-evaporated-sterilized milk, was fed in comparison with the original samples. In all cases the milk-ash showed less ossifying potency than the milks from which they were obtained, indicating that a part of the calcifying properties of the various milk samples must have been due to the presence of vitamin D.

The data obtained indicate that the milks described are not rich in vitamin D. While commercial methods of evaporation and sterilization evidently affect the ossifying potency of milk to an appreciable degree, no attempt has been made to differentiate between mineral loss and vitamin D destruction.

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THE RELATION OF THE FAT-SOLUBLE VITAMINS (A AND D) TO THE DEVELOPMENT OF EXPERIMENTAL RICKETS IN RABBITS

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EXPERIMENTAL results have been at some variance as regards the etiological interdependence of the fat-soluble vitamins with calcium and phosphorus in rickets. The production of rickets in puppies by a diet deficient in fat-soluble vitamins (Mellanby) followed by a demonstration of the relationship of this factor to calcium and phosphorus metabolism (McCollum, Simmonds, Shipley and Park; Sherman and Pappenheimer; Korenchevsky), has stimulated attempts to define the relative importance of these factors in the production of experimental rickets.

Mellanby concluded that rickets developed in puppies whose diets were deficient only in the antirachitic fat-soluble organic factor. Attempts to produce rickets in guinea pigs (Tozer), kittens (Mackay), and pigs (Zilva, Golding, Drummond and Coward), by feeding a diet deficient only in the fat-soluble vitamins, failed. Korenchevsky and also Goldblatt produced rickets in rats by a diet deficient only in the fat-soluble vitamins, although Shipley, Park, McCollum and Simmonds, and Hess, McCann and Pappenheimer concluded that there must be a deficiency both of the fat-soluble vitamin and of calcium or phosphorus to produce rickets in rats.

Rickets has been produced in the rabbit by diets deficient in fat-soluble vitamin, low in phosphorus, and high in calcium (Goldblatt and Moritz). It was considered of interest then to observe the effects of a diet deficient only in the fat-soluble vitamins (A and D) on the degree of calcification of the bones and the development of rickets in rabbits and at the same time to repeat the experiment on rats.

Experimental

Diet. The basal diet used was essentially the same as that used for the production of rickets in rats by Korenchevsky and by Goldblatt. This diet was eaten readily by the rabbits; only 4 of the 30 observed refused to eat.

Each animal received daily 5 grams of alcohol- and ether- extracted

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and dried alfalfa hay (Goldblatt and Moritz) to supply roughage, and 7.5 cc. of decitrated lemon juice to supply the antiscorbutic factor.

The animals were divided into four groups as follows. Group I received a normal diet consisting of milk, oats, lettuce, cabbage, and alfalfa hay. This diet will be referred to as (N); Group II received a complete diet consisting of the basal diet in which the salt mixture used was McCollum's No. 185. This diet contained 0.6 per cent of Ca (determined) and 0.8 per cent of phosphorus (calculated). With a daily intake of 5 grams of alfalfa hay containing 2.14 per cent of calcium (Forbes) an adequate calcium intake was assured. Each animal received 10 drops (about 220 milligrams) of potent cod liver oil daily. This diet will be referred to as (−A+CLO). Group III received a complete diet except for fat-soluble vitamin, and was the same as diet (−A+CLO), but without the cod liver oil. This will be referred to as diet (−A). Group IV received diet (−A−P), deficient in phosphorus and fat-soluble vitamin, and consisted of the basal diet in which a salt mixture devoid of phosphorus (No. 6 of Korenchevsky) was used.

Animals. Litters of young rabbits varying in age from 33 to 47 days were used. Each litter was divided in such a manner that comparable animals would be in each dietetic group. Four were placed in group I, eleven in group II, eleven in group III and two in group IV.

All animals were weighed twice a week. The amount of food consumed daily by each animal was noted. Two animals belonging to the same group were kept in each cage in a room receiving no direct sunlight.

After varying periods of observation on the diet, comparable animals were selected from each group and killed by bleeding from the carotid artery.

Blood Chemistry. The determination of calcium by the Tisdall modification of the Kramer-Tisdall method and of inorganic phosphorus by the Tisdall method were made on the blood serum obtained at the time of death.

Bone Analysis. The right and left radii removed at the time of autopsy were carefully cleaned of all adherent soft tissue and weighed separately. The bones were then dried for 12 hours at 110° C, weighed again and ashed. The ash was dissolved in 2 cc. of concentrated hydrochloric acid, made up to a volume of about 75 cc. with distilled water in a 100 cc. volumetric flask and neutralized with ammonium hydroxide. Acidification was then brought about with an excess of 5 drops of concentrated hydrochloric acid, water was added to bring the volume up to 100 cc. and this solution thoroughly mixed.

Aliquots of 2 cc. were then pipetted into each of two narrow tipped 15 cc. centrifuge tubes to which were added 1 cc. of a 20 per cent solution of sodium acetate and 1 cc. of a saturated solution of ammonium oxalate. After standing for one hour the calcium oxalate was separated by centrifuging 15 minutes at high speed and washed twice with 2 per cent ammonium hydroxide, as in the Kramer-Trisdall method for serum calcium.

The washed calcium oxalate was dissolved in 3 cc. of N/1 sulphuric acid and titrated against potassium permanganate which had been standardized against N/50 sodium oxalate.

Two determinations of calcium were thus made on each bone, and from these figures and those of the bones, before and after drying, the percentages of water and of calcium per wet and dry weight of bone were calculated.

Histological Examination. The sixth, seventh and eighth ribs from the right side were fixed in formalin, decalcified in Muller's fluid and stained with hematoxylin and eosin.

X-Ray Examination. X-ray photographs were taken once a week on animals 1386, 1387, 1391, 1393 and 1400.

All observations and data on these animals, except the growth curves and x-rays are recorded in Table I. There is a definite reduction of the serum calcium of the rabbits in group III, on (−A) diet, while there are no significant differences in the amounts of serum calcium in the other three groups. The inorganic serum phosphorus of rabbits in group IV is greatly reduced but is normal in the other three groups.

In groups I, II and III there are no consistent differences in the percentages of calcium in the bones. However, the average percentage of calcium in the bones of group III is decreased per wet weight as compared with those in groups I and II. The figures show that the amount of calcium per whole bone is reduced in group III, but the number of observations is too small to determine, on the basis of statistical analysis, that the diminution in the average percentage of calcium per net weight represents a real decrease in the degree of calcification of the bones of rabbits on diet (−A).

The rabbits in group IV show a definite reduction in the percentage of calcium per wet and dry weights of the bones.

Both gross and histological examination of the bones of the animals in group I showed them to be normal, while those of group II were normal or slightly osteoporotic. There was neither gross nor histological evidence of rickets in any of the animals of group III, although these animals were killed and examined after having been on the diet from 14 to 43 days. There were varying degrees of osteoporosis. At the end of the

TABLE I

Rabbit No.	Initial Age in Days	Days on Diet	Weight in grams		Blood mgm. per cent		Bones	
			Initial	Final	Ca	P	Ca Per cent Wet wt.	Micro. Exam.
Group I (Normal Diet)								
1499	49	16	1135	1340	17.25	8.76	11.86	Normal
1498	64	24	1312	1460	17.81	8.00	13.43	Normal
1500 A	50	24	867	1075	15.49	5.63	10.47	Normal
1495	41	33	612	1170	16.21	9.15	10.75	Normal
(Averages)					—	—	—	
Group II ((−A+CLO) Diet)								
1395	40	31	360	625	12.69	8.94	8.36	Normal
1409	33	31	660	885	16.89	6.41	9.26	Normal
1496	39	35	562	920	13.56	10.61	9.53	Osteoporosis
1497	39	35	597	1020	16.32	8.70	9.48	Normal
1408	33	40	579	1115	16.81	8.42	11.82	Normal
1392	43	41	425	830	12.74	9.09	9.79	Normal
1397	37	41	477	1050	14.58	8.10	11.35	Osteoporosis
1504	47	42	770	1270	12.46	8.27	9.96	Osteoporosis
1505	47	42	780	1360	15.08	7.45	11.66	Osteoporosis
1393	41	45	652	1265	15.09	8.78	11.85	Normal
1394	41	45	575	1297	16.98	8.42	12.00	Normal
(Averages)					14.83	8.47	10.46	
Group III ((−A) Diet)								
1500	47	14	870	735	10.75	8.76	7.86	Osteoporosis
1389	40	31	379	485			8.08	Osteoporosis
1390	40	31	404	540	10.15	8.85	6.81	Normal
1410	33	32	632	810	14.35	6.48	10.27	Normal
1502	47	32	815	995	8.03	8.79	10.02	Normal
1493	39	35	643	625	7.48	9.15	7.50	Osteoporosis
1391	37	41	472	635	8.04	9.39	8.04	Osteoporosis
1386	43	41	447	580	9.49	5.46	8.04	Osteoporosis
1503	47	42	750	780	8.24	6.79	8.41	Osteoporosis
1501	47	42	680	830	8.24	9.37	9.65	Osteoporosis
1387	41	43	617	713			10.35	Osteoporosis
(Averages)					9.41	8.11	8.63	
Group IV ((−A−P) Diet)								
1400	37	45	574	955	16.86	4.34	7.47	Rickets
1399	41	47	609	1020	15.43	2.75	7.23	Rickets

experimental period some of these animals were still gaining weight, some had stopped gaining and others were on the decline. The animals in group IV showed moderate rickets.

Chart 1 contrasts the composite curves of the weekly weight increases of the rabbits in group II and III.

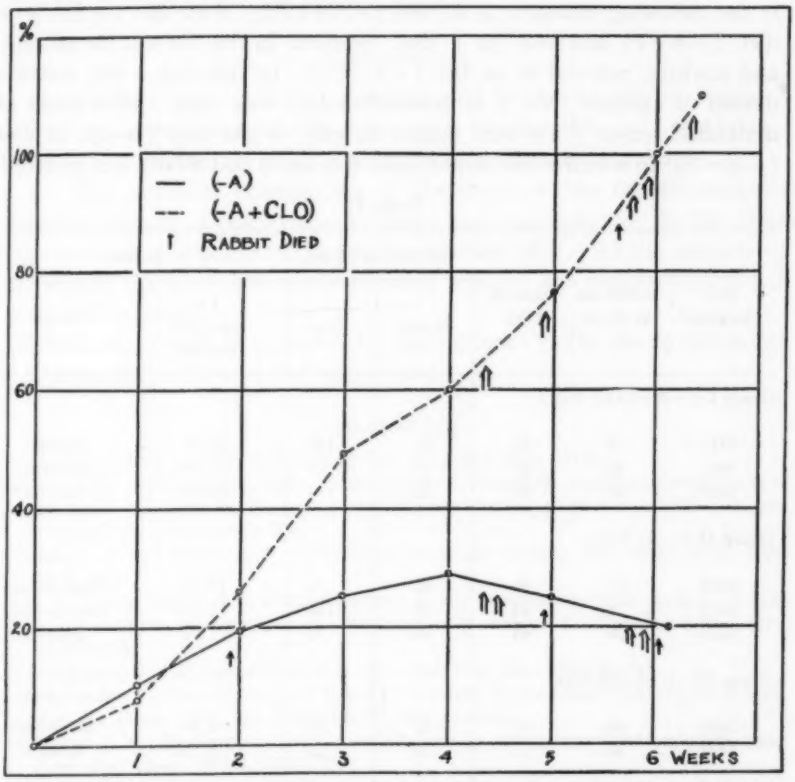

CHART 1.—Composite curves of percentage weight increase in eleven rabbits on diet (−A) and eleven on diet (−A+CLO).

Weekly x-ray photographs of rabbits 1386, 1387 and 1391 on diet (−A) showed no evidence of rickets nor did rabbits 1392 and 1393 on diet (−A+CLO). Rabbit 1400 on diet (−A−P) showed distinct changes at the end of the second week indicative of rickets which became progressively more marked and consisted in the development of a rachitic metaphysis separating the epiphysis and diaphysis seen at the distal end of the

radius, ulna, humerus, tibia, fibula and femur. There was cupping and imperfect calcification of the diaphysis as well as deficient calcification of the epiphysis.

Experiments on Rats.

As a dietary control 9 rats were placed on the same diets fed the rabbits in the following manner: 3 on diet (−A+CLO); 3 on diet (−A); 2 on diet (−A−P) and one on a diet deficient in the fat-soluble vitamin and calcium, referred to as diet (−A−Ca). In this diet a salt mixture devoid of calcium (No. 2 of Korenchevsky) was used. Five parts of decitrated lemon juice were added directly to the diet (except in diet (−A−Ca) in which whole lemon juice was used) and alfalfa was omitted.

Table II

Rat Number	Initial age in Days	Days on Diet	Weight in Grams		Bones	
			Initial	Final	Ca per cent Wet wt.	Micro. Exam.
Group I ((−A+CLO) Diet)						
1417	38	45	46	138	14.24	Normal
1418	38	45	63	122	13.49	Normal
1419	30	45	23	70	10.95	Normal
Group II ((−A) Diet)						
1414	38	44	46	82	10.53	Osteoporosis
1415	38	44	71	117	11.36	Osteoporosis
1416	30	44	38	70	7.92	Rickets
Group III ((−A−P) Diet)						
1420	30	37	42	83	8.19	Osteoporosis
1421	30	37	36	64	5.56	Rickets
Group IV ((−A−Ca) Diet)						
1422	30	36	42	62	6.65	Rickets

Calcium analysis was made on femur, fibula and tibia from both legs of each animal and histological examination was made on the ribs.

Table II shows that the percentage of calcium in wet bones of rats on diet (−A) was distinctly lower than in those on diet (−A+CLO) although not so low as in the rats on diet (−A−P) or (−A−Ca).

Histological examination showed the bones of rats on diet (−A+CLO) to be normal. There was definite rickets in rat 1416 on diet (−A) while the other two showed only osteoporosis. The percentage of calcium in both wet and dry bones of rat 1416 was markedly decreased. Of the two rats on diet (−A−P), one showed severe rickets and the other severe osteoporosis. The rat on diet (−A−Ca) showed rickets.

Conclusions

1. Rabbits fed on a diet deficient only in the fat-soluble vitamins (diet −A) showed no evidence of rickets at the end of from 14 to 45 days.
2. The blood serum calcium was almost uniformly decreased.
3. The chemical examination of the bones of the rabbits suggests that the amount of calcium in the whole bone was reduced. In the three rats on the diet deficient in fat-soluble vitamins (diet −A) the amount of calcium in the bone was unquestionably reduced and rickets occurred in one of the animals.

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THE VITAMIN A CONTENT OF YELLOW AND WHITE-CAPPED YELLOW DENT CORN*

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In 1919, Steenbock (1) called attention to the association between certain yellow pigments and vitamin A in nature and formulated a working hypothesis which made the assumption that "the fat-soluble vitamin is a yellow plant pigment or closely related compound." A short time before this, Drummond (2) had reported vitamin A to be absent from crystalline carotin (carrotene) but that a crude carotin fraction contained slight traces of the vitamin. Later, Rosenheim and Drummond (3) showed that fat-soluble A is not identical with carotin or xanthophyll. Also, Stephenson (4) found that carotin does not have the properties of vitamin A. More recently Widmark (5) has reported that plants which lose ability to form chlorophyll or lipochrome also are unable to form vitamin A. In 1928, v. Euler, v. Euler, and Hellström (20) presented data which indicate that carotin preparations have a high vitamin A potency, although their contention is disputed by Duliere, Morton and Drummond (21), and a recent report (1929) by Collison, Hume, Smedley-MacLean and Smith shows that carotin from cabbage fat has high vitamin A potency (22).

Soon after the suggestion of the above relationship by Steenbock, Palmer (6) called attention to exceptions to the hypothesis, the basis for the exceptions being chiefly the results of his investigations (7) and that of himself and Kempster (8) on the relation of plant carotinoids to the animal organism. Also Drummond and Coward (9) failed to find the association noted by Steenbock to prevail in all cases.

A study of a variety of substances by Steenbock and his associates (10, 11, 12, 13, 14,) confirmed the general relationship between the yellow pigments and the presence of vitamin A but they noted that the amount of vitamin does not always parallel the pigment content. The suggestion was made (1, 12) that possibly the pigment is in a leuco form in cases in which the vitamin content is high and the pigmentation slight. Such a

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

suggestion would apply in the case of the colorless fats noted by Drummond and Coward (9) and Palmer, Kennedy and Kempster (15). The latter authors review and discuss critically the data and the hypothesis concerning the relationship of yellow pigments to vitamin A.

Of particular interest in the present report is the comparison of the vitamin A content of white and yellow maizes by Steenbock and Boutwell (10), who showed the vitamin to be present in the yellow variety but not in demonstrable amounts in the white. Also, numerous practical feeding trials have demonstrated the superiority of the yellow variety as a source of vitamin A.

In view of the local production of white-capped, yellow dent corn it was of interest to determine quantitatively the amount of vitamin A in this variety as compared with that of the yellow variety, for the information of the stock feeder. There was also in mind the possibility that the data might be an addition to that already existing on the relation of vitamin A to pigments.

Experimental Procedure

The procedure for the determination of vitamin A was essentially that outlined by Sherman and Munsell (16) except where otherwise indicated. The breeding colony ration was Sherman's No. 13, consisting of 2 parts ground whole wheat, 1 part whole milk powder and 2 per cent of the weight of the wheat as sodium chloride (17), modified by the incorporation of 10 per cent meat scrap (Swift's) at the proportionate expense of the wheat and milk. Young white rats, weaned at 28 days of age and weighing between 35 and 55 gm., were placed upon a basal, vitamin A-free diet, which was essentially that described by Sherman and Munsell, Diet No. 380, but modified to include the antirachitic factor. It was composed of purified casein 20 per cent, Osborne and Mendel (18) salt mixture 4 per cent, sodium chloride 1 per cent, dried yeast 10 per cent, corn starch 63 per cent, and 2 per cent of olive oil, containing 25 mg. cholesterol per cc., the oil and dissolved cholesterol having been irradiated with a quartz mercury-vapor lamp for 30 minutes at 60 cm. The vitamin B complex was determined in the yeast and if sufficiently potent the amount was decreased to 5 per cent and the corn starch increased correspondingly.

During the pre-test period the animals were kept in groups but during the test period they were confined in individual cages. After transfer to the individual cages, yeast and the olive oil solution of cholesterol were removed from the basal diet and fed as separate supplements, 300 mg. to 500 mg. of the former, depending upon its potency, and 5 drops of the latter

per day (except Sunday). Cornstarch replaced these two ingredients in the basal diet.

The animals were assigned to the test and control groups so that litter mates appeared in each group. Insofar as possible an equal distribution of sexes was made.

The depletion period averaged 5 to 6 weeks. An individual was ready for the test period when growth had definitely ceased, no gain made in 3 to 5 days, and it was considered desirable to have some evidence of ophthalmia. In practically every case an eye disturbance was observed which varied from the whitening of the edge of the lid and loss of the usual beady appearance to a complete closure of the eye with marked reddening. The opthalmias were cured during the first week of the test period.

The material under examination was ground fine and fed as a daily supplement (except Sunday). On the basis of preliminary feeding trials it was estimated that 500 mg. of the yellow corn per rat per day and 750 mg. of the white-capped, yellow dent variety would allow an average gain of 3 gm. per week for an 8-week experimental period. For the white-capped, yellow dent variety a local strain of Mercer White-Capped, Yellow Dent was used, and a supply of yellow dent from a local dealer for the yellow variety.

Discussion

The average net gains for the eight-week test period were 34 gm. (P.E. $\pm$ 2.9) for the group of 10 animals fed the white-capped yellow dent variety, and 40 gm. (P.E. $\pm$ 1.5) for the group of 11 fed the yellow variety. The difference between the two means, 6, is not quite twice the probable error, 3.2, of the difference between the two means (19). Hence the difference is not significant and the growth response is considered practically identical for the samples. Table I displays a summary of the results.

Since the same growth response was obtained with 500 mg. of the yellow variety as with 750 mg. of the white-capped, and both responses were less than normal, the yellow variety may be said to be about 50 per cent more potent with reference to vitamin A than the white-capped.

Although this information is available only in the case of a laboratory test animal, the white rat, it is suggested that when corn is relied upon as a source of vitamin A in a livestock ration, more of the white-capped variety be used than of the yellow, especially if the amount of the latter recommended is not much more than enough to meet the vitamin A requirements. The meal obtained by grinding white-capped yellow dent corn is of a lighter yellow color than that of the yellow dent. These findings are

in accord with those of Steenbock and Boutwell (10). The pigmentation of the white-capped variety is less than that of the yellow but greater than that of the non-pigmented, white variety, the last named variety being very deficient in the A factor according to Steenbock and Boutwell. The vitamin

TABLE I

SUMMARY OF RAT GROWTH RECORDS, VITAMIN A TEST

Supplement	No. of animals	Av. Weight beginning of test period gm.	Av. Weight end of test period gm.	Av. Gain eight wk. test period gm.	Difference and the probable error
500 mg. yellow dent corn	11	93	133	40 (P.E.*±1.5)	
750 mg. white-capped yellow dent	10	94	128	34 (P.E. ±2.9)	6±3.2
Controls, no supplement	13	95	Av. wt. at death—77 gm. Av. survival period—19 days		

$$*\text{P.E.} = 0.6745\sqrt{\frac{\sum d^2}{n(n-1)}}$$

A content of the white-capped variety is intermediate between that of the yellow and white varieties.

CONCLUSIONS

1. Yellow dent corn was found to be about 50 per cent more potent with reference to vitamin A than a white-capped yellow dent variety.

2. The more highly pigmented variety contains the greater amount of vitamin A.

3. When the white-capped variety is used in compounding a ration, consideration should be given to the lower vitamin A content, as compared with the yellow dent variety.

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THE NEPHROPATHOGENIC ACTION OF CYSTINE
II. THE DIETARY CONTROL OF CYSTINE NEPHROSIS

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Introduction

In A recent contribution from this laboratory Cox, Smythe, and Fishback (1) have shown that "young rats of 60 gm. or less develop acute toxic nephrosis when restricted to synthetic diets containing 0.3 to 0.9 per cent of free cystine." The observations made on loss of appetite and weight, enlarged kidneys, death of some rats, and recovery of others without change of ration, were similar to those of Hartwell (2) using rations containing 20 per cent of edestin. Hartwell suggested that some specific amino acid was responsible for the indisposition of the rats and our work indicates that cystine may be the causative agent. She found that if the vitamin B of the diet, supplied by marmite, were increased sufficiently the symptoms of the disease did not appear.

Hartwell's success in preventing kidney injury due to edestin suggested the experiments reported in this communication in which we have studied the effects of a varying supply of yeast extract on the development of cystine nephrosis.

We have used the Diet C of Cox, Smythe, and Fishback as our basal ration. (See Table I.) To produce nephrosis, we have used Diet 3 in which 0.3 per cent of cystine replaces an equal amount of the casein

Table I

Composition of Diets

	Diet C. per cent	Diet 3. per cent
Casein	15.0	14.7
Cystine	0.0	0.3
Dextrin	40.0	40.0
Sucrose	15.0	15.0
Lard	19.0	19.0
Cod liver oil	5.0	5.0
Salt mixture*	4.0	4.0
Agar agar	2.0	2.0

* Osborne and Mendel (3).

of Diet C. It is worthy of note that the casein that has been used throughout these studies of cystine nephrosis has been a commercial casein labeled "Free from Water-Soluble Vitamin-B" supplied by the Harris laboratories. For yeast extract we have used the Osborne and Wakeman (4) vitamin-B fraction of yeast (Yeast Vitamin, Harris), supplied in two different ways. It has been fed separately in the form of pills made with 50 mg. of the yeast concentrate and 100 mg. of dextrin. In other experiments it has replaced differing amounts of dextrin in the ration.

We have encountered variable resistance of rats to the effects of cystine and have attempted to determine whether it is inherent, or due to dietary influences previous to the experimental period. One group of rats from a commercial source did not show symptoms of nephrosis within ten days. Another group, obtained from another laboratory, had been born and reared on a ration containing 20 per cent of dried brewer's yeast. The rats of this latter group showed symptoms of a distinctly milder order than the rats from our colony, bred and raised on a normal stock ration.

Our procedure has been as follows. When the rats of a litter have attained body weights of about 35 gm. they are placed in individual cages and offered the experimental diet, *ad libitum*. They are weighed daily but the food intake is determined only for the total five day period. After the fifth day both food and rats are weighed daily. At the end of ten days they are killed and the kidneys removed and weighed. These kidney weights are compared with those of Donaldson (5) and those of the other rats of the litter.

In some of our first experiments the above observations were considered sufficient. But in the case of the rats in which it was desired to study the intensity of the disease, the non-protein nitrogen of the blood at death was determined by the procedure previously used (1). All kidneys were preserved in 10 per cent formaldehyde and many of them were prepared for histological examination.

Experimental

As all the data of this investigation are interrelated we have condensed them to average values which we present in Table II. The response of both sexes to the diets was apparently the same. Rats from one litter were distributed among the related groups. In the columns under the heading of "Kidney Weights" we have considered the weights of Donaldson as 100 per cent. Our figures are based upon the maximum weight attained by the rat.

Our first experiment tested whether increasing the number of pills

TABLE II

EFFECTS ON GROWTH, FOOD CONSUMPTION, KIDNEY WEIGHTS, AND NON-PROTEIN NITROGEN OF THE BLOOD OF A VARYING SUPPLY OF YEAST EXTRACT IN THE DIET OF YOUNG RATS CONSUMING FREE CYSTINE

Group	Yeast Extract Daily mg.	Diet	Number of rats	Average Body Weight			Food Consumed		Kidney Weights per cent of normal		Average Final blood Non-Protein N mg. per 100 cc.
				Initial gm.	Maximum gm.	Final gm.	First five days gm.	Second five days gm.	Variation	Average	
I	50	C	5	37.4	56.8	56.8	23.0	26.4	92–110	100	
II	0	3	4	36.8	52.0	50.6	23.3	16.6	167–246	204	
III	50	3	11	36.2	54.0	49.8	23.8	17.2	98–240	178	
IV	100	3	4	36.8	59.8	57.0	27.2	19.7	95–204	157	
V	150	3	4	37.0	65.0	62.8	27.8	24.5	104–221	166	
VI	300	3	4	37.8	73.3	73.3	24.4	33.1	104–117	111	
VII	0	1–V	8	37.1	53.8	48.8	24.1	16.0	107–220	177	165
VIII	0	5–V	6	36.3	68.5	68.5	25.4	32.6	87–103	93	36
IX	0	10–V	5	35.2	75.0	75.0	26.4	35.8	94–107	101	39
X	50	C	4	41.5	63.0	63.0	25.1	29.2	95–100	98	55
XI	50	3	6	36.5	56.0	55.9	21.6	19.1	105–156	125	74
XII	150	3	4	32.0	62.2	62.2	20.3	26.2	105–126	115	51
XIII	300	3	4	33.5	68.5	68.5	19.7	27.7	96–132	115	53
XIV	50	C	7	37.7	53.4	53.4	24.3	25.7	76–100	89	80
XV	50	3	9	36.0	52.9	51.5	22.3	19.3	87–198	135	138
XVI	50	C	8	35.9	50.0	50.0	20.6	22.2	85–108	95	53
XVII	50	3	11	36.6	50.8	49.9	21.1	18.2	93–222	158	121

containing 50 mg. of yeast vitamin (Harris) would prevent nephrosis. Group I (see Table II) was on the casein diet without added cystine. Group II, receiving no yeast extract, served as control. Other Groups, III, IV, V, and VI received daily 1, 2, 3, and 6 pills representing 50, 100, 150, and 300 mg. of the yeast extract. The latter was about the limiting amount that would be ingested by the rats and frequently one or two of the pills would not be consumed.

The rats of Group I did not grow rapidly due to lack of cystine in the ration but no symptoms of kidney disorder appeared. Rats of Groups II to V inclusive developed nephrosis as indicated by loss of weight and appetite during the second five day period and by kidney hypertrophy. The symptoms were progressively less intense as the yeast extract supply was increased. Four rats of Group III and two rats of Group IV died on the ninth day of the experiment, all showing the typically enlarged kidneys. Rats of Group VI receiving 300 mg. of the yeast extract showed no symptoms of the disease.

The rats of Groups V and VI at times did not consume the entire supply of the yeast extract pills especially during the first three or four days. To meet this exigency the powder was incorporated in Diet 3 replacing an equal amount of dextrin. These rations which contain 1, 5, and 10 per cent of the yeast extract are represented by Diets I-V, 5-V, and 10-V respectively. The rats of Group VII were not protected from the cystine, but those of Groups VIII and IX showed no symptoms. Based on the food intake of the first five days these latter rats received respectively about 250 and 500 mg. of yeast powder daily. Group I received about 50 mg. Two of the rats of Group I died on the ninth day of the experiment. Blood was not obtained from them and 165 mg. represents the non-protein nitrogen of the blood of the six survivors.

The rats of Group X to XIII inclusive were born and reared to the initial weights shown in the table on a ration containing 20 per cent of dried brewer's yeast. They were also from different stock than the rats of the preceding groups. Their evident resistance to the effects of free cystine in the diet led us to breed rats from our own stock but fed a ration containing 20 per cent of brewer's yeast. The rats of Groups XIV and XV were from three litters born and reared on the stock ration shown in Table III. The rats of Groups XVI and XVII are from three litters from the same parents as Groups XIV and XV but supplied throughout the breeding, gestation, and lactation periods with the yeast ration of Table IV.

Cystine nephrosis developed as usual in the rats of Groups XV and XVII ingesting free cystine, indicating that the diet previous to the ex-

perimental period has little or no influence on the susceptibility of the rats to the disease. Resistance may be hereditary but more data than we present are necessary for proof.

Examination of the kidneys of representative rats of the various groups revealed lesions, in general, the same as those reported by Cox, Smythe, and Fishback (1).

TABLE III

STOCK RATION

	per cent
Alfalfa	10
Meat scraps	12
Casein	12
Sodium chloride	1
"Red Dog" Flour	65

To the above diet 20 gm. of dried brewer's yeast, 20 gm. of cod liver oil, and 1 cc. of a 0.1 per cent solution of potassium iodide per kilogram were added. Milk was fed *ad libitum*. Each rat received 2 gm. of compressed baker's yeast daily, and 1 dog biscuit and 40 gm. of lettuce twice a week.

TABLE IV

YEAST RATION

	per cent
Casein	30
Yeast	20
Dextrin	46
Salt mixture*	4
Wheat germ oil, 5 drops daily	
Cod liver oil, 5 drops daily	

* Osborne and Mendel (3).

DISCUSSION

It is apparent from the data presented in Table II that there is some substance present in the Osborne and Wakeman vitamin B concentrate of yeast that protects young rats from cystine nephrosis. The active factor may play a very special role in the metabolism of cystine or the excretion of that amino acid or its metabolic products. On the other hand, it may be necessary for the disposal of amino acids in general.

Hartwell's (2) experience in preventing kidney injury due to edestin by the use of autoclaved marmite indicates that the active substance is not identical with the antineuritic factor. The very special manifestation of the deficiency, the rapid development of the symptoms, the brief period of the rat's life during which susceptibility occurs, and the recovery of rats without any change in diet indicate that the active factor is not identical with any of the known accessory food substances.

Difference of opinion exists relative to the development of renal injuries due to high protein diets. As Mitchell (6) has pointed out recently, those investigators who have had positive nephritic findings have generally employed diets that were not demonstrably complete. Maclean, Smith, and Urquhart (7) have shown that rabbits on diets from which green food is excluded become nephritic and that the inclusion of cabbage leaves in the diet, which may be very high in protein, prevents kidney injury or cures it if it has developed. They have suggested that "fresh vegetables may be more important than the usual abstention from protein food" in the treatment of nephritis. Our observations on the efficacy of yeast extracts in nullifying the nephrotoxicity of cystine suggest the possible use of such active extracts in the treatment of human nephritis.

The large amount of yeast extract that we have found necessary as a preventative dose indicates either that yeast is not particularly rich in the active factor, or that the Osborne and Wakeman procedure does not concentrate it. Definite proof of the individuality of the factor which prevents the cystine nephrosis must await the exclusion of other known dietary factors, especially the anti-pellagric.

Summary.

Cystine nephrosis in very young rats may be prevented by the inclusion in the diet of sufficient of the Osborne and Wakeman vitamin B concentrate of yeast.

The active substance of yeast extract which protects rats from cystine nephrosis is probably distinct from any of the known accessory food factors.

The susceptibility of young rats to cystine nephrosis is probably hereditary.

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A STUDY OF THE ANEMIA OF YOUNG PIGS AND ITS PREVENTION*

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EVERYONE connected with the swine industry recognizes that there is a tremendous loss in pig raising due to the large number of deaths which so often occur during the early growing period. Data show that about 35 per cent of the pigs farrowed are lost during the nursing period. There are many factors which probably influence this excessive death rate, but undoubtedly one factor is the large prevalence of anemia in suckling pigs. The recognition of anemia in suckling pigs is recent and the detailed facts regarding its cause and prevention are rather meager.

McGowan and Crichton (1, 2) were pioneer investigators in this field. They not only observed the presence of anemia in suckling pigs when confined indoors, but also studied the symptoms of this disease and made suggestions as to its prevention. These workers noted that anemia was exceedingly prevalent in British pig breeding establishments where the sows were brought into houses, put in pens with concrete floors, and fed a dry ration during the farrowing period. The sows would farrow normally but when the pigs were from three to four weeks old, they took on a "stocky" appearance due to oedema of the skin. The breathing became pumping in character and "thumps," a spasmodic jerking of the diaphragm, developed. Death was common and often entire litters perished. On post-mortem examination, the heart was found to be greatly dilated, there was an excess of pericardial fluid, and the lungs were oedematous with effusion into the pleural cavities. The blood was extremely watery and pale, the hemoglobin often being as low as 15 per cent of the normal. In the cases which continued to live, the animals became very emaciated, ceased to grow, and

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lost their appetites. Diarrhoea was present in a large number of the pigs.

McGowan and Crichton believed the chief cause of this disease to be an iron deficiency. When the sows were out of doors they obtained abundant iron from the grass and the soil, but when they were kept indoors, owing to the concrete floors and the nature of the food, the supply of iron decreased. Excellent results were obtained both in the amelioration of the anemia from which the pigs were already suffering and in its prevention in the younger pigs when large doses of ferric oxide were fed to the sow. The British workers were in doubt as to whether the beneficial effect noted in the young pigs when the sows were fed ferric oxide was due to a more abundant source of iron in the refuse and feces of the sow, or to an increased iron content of the sow's milk. They suggest that the effect is probably not due to any change in the composition of the milk.

This specific question was one of the reasons which prompted us to investigate the effect of diet on the iron content of milk. We were unable to make this investigation on sow's milk but we did find that increasing the iron content of the rations fed to cows and to goats did not increase the iron content of the respective milks (3). Undoubtedly this fundamental fact also can be applied to the pig.

Doyle, Mathews, and Whiting (4) have observed the same condition as reported by McGowan and Crichton in young suckling pigs reared by sows kept indoors. These workers have made an excellent study of the symptoms of this disease, including both the gross and microscopic pathological changes. They concluded that the anemia was not influenced by the vitamin supply in the ration nor by the addition of ferric lactate, but it was markedly affected by some factor in outside conditions. Although they did not analyze the out-of-door factor which played an important part in hemoglobin formation, they have demonstrated by recent work (5) that irradiation by means of a quartz mercury vapor lamp has no beneficial effect on the hemoglobin content of the blood of young pigs.

An extensive study of the factors affecting hemoglobin synthesis in several species of animals when restricted to milk diets has been underway at this station during the past five years. Definite knowledge of these factors was gained when it was demonstrated that copper is essential as a supplement to iron for normal hemoglobin synthesis (6).

With this fundamental fact in mind we wished to extend our studies to the pig in addition to the rabbit, the chick, and the rat. We have demonstrated that the rabbit and the rat must be restricted to a milk diet for some time after the normal weaning period in order to produce a severe anemia. We were interested, therefore, in determining if the pig differs from these

animals and is susceptible to severe anemia during the suckling state. If this is true, then a study of the factors which will prevent this condition is of much value. The effect on the hemoglobin content of the blood of suckling pigs, of feeding iron and copper salts to the sow during the gestation period, and to the young after their birth, is an important question to be answered.

Experimental

Work with Pigs after Weaning Period

The first part of this study consisted of following the hemoglobin content of the blood of pigs placed, shortly after weaning, on a diet quite low in iron. Iron and copper additions were also made to this ration comparatively low in iron, to determine if the hemoglobin content could be modified by these salts. Thirty gilts weighing from 40 to 60 pounds were selected from the general herd of pigs at the University swine barn. They were started on the experiment June 19, 1928, which was about two weeks after most of the pigs had been weaned. The thirty gilts were divided into ten lots of 3 pigs each in such a manner that each lot contained one Poland China, one Duroc Jersey, and one Chester White pig. All the groups were kept indoors and each lot placed in a pen with concrete floor and partial wooden overlay. The pens were equipped with a concrete trough for feeding purposes.

The basal ration consisted of yellow corn 99 parts, common salt 1 part, and skim milk; 3 pounds of the latter were fed for each pound of grain mixture. Each pig was also allowed 50 cc. of cod liver oil per week. One lot received only the basal ration while varying amounts of iron and copper additions were fed to the other lots. The additions made to the rations fed each of the groups were as follows:

Group 1, Basal
Group 2, Basal plus 10 mg. iron per pig daily
Group 3, Basal plus 50 mg. iron per pig daily
Group 4, Basal plus 250 mg. iron per pig daily
Group 5, Basal plus 2 mg. copper per pig daily
Group 6, Basal plus 50 mg. iron and 2 mg. copper per pig daily
Group 7, Basal plus 50 mg. iron and 10 mg. copper per pig daily
Group 8, Basal plus 50 mg. iron and 20 mg. copper per pig
Group 9, Basal plus 50 mg. iron and 50 mg. copper per pig daily
Group 10, Basal plus Fe_2O_3, 0.5 pound per 100 pounds grain mixture and 2 mg. copper per pig daily.

All the iron additions except in Lot 10 were made as ferric sulphate, $Fe_2(SO_4)_3$, and all the copper additions were made as copper sulphate $CuSO_4$. The salts were dissolved in water and made to definite volumes. Amounts of these solutions equivalent to the quantity of iron and copper to be fed were added to the milk daily.

Weights of the animals were taken weekly. Hemoglobin determinations were made at the beginning of the experiment and every two weeks thereafter during the first part of the experiment, and every month during the latter part of the study. The blood was obtained by tail bleeding and the hemoglobin determined by the Newcomer method.

The results of the hemoglobin determinations are given in Table I. Each figure is the average of the hemoglobin values for the three sows in one lot.

A study of the figures shows that the great majority of them fall between 8–10 grams per 100 cc. of blood. This range is undoubtedly the normal hemoglobin value for pigs. The hemoglobin values for the three sows receiving the basal ration alone are well within this range. This ration, although low in iron, must furnish a sufficient amount, together with what may be picked up in the refuse, to allow normal hemoglobin building during the period of growth following weaning. If these pigs had been anemic during the suckling stage, they had improved enough by the time the experiment was started to show a normal blood stream. The hemoglobin content of the blood from sows receiving varying additions of iron and copper was no higher than that of the sows receiving the basal ration. The sows fed the Fe_2O_3 gave hemoglobin values slightly lower than the other sows. We have no explanation for this result. Another interesting fact is that the hemoglobin values for all groups were the lowest during the second month on the ration, which is probably due to the rapid rate of growth during this time.

We used all the sows in this experiment because then we could study the effect of these additions upon the hemoglobin values in the young produced by the sows on the various rations. The sows were therefore bred during the winter so that the young would be farrowed during April and May.

Work with Pigs During the Suckling Period

The first sow to farrow was No. 520 in Group 1. We present the curves for the hemoglobin values of two of the eleven pigs in this litter in Chart 1. These curves are typical of the blood changes which took place in all the pigs of this litter. Every pig in this litter which received no addition to the mother's milk was dead within seven weeks.

TABLE I

THE EFFECT OF IRON AND COPPER ADDITIONS TO A BASAL RATION FED TO GROWING SOWS.
Sows were started on the different rations shortly after weaning.
*Grams Hemoglobin per 100 cc. Blood

No. of ration	Days on Ration									
	0	14	28	40	74	105	138	167	216	Average
1	8.78	9.07	7.53	7.33	7.76	8.58	8.47	11.05	9.80	8.7
2	7.98	9.08	7.65	7.58	7.78	7.92	9.53	8.41	10.23	8.7
3	8.67	9.45	7.70	7.34	7.05	8.44	7.72	9.81	10.77	8.8
4	8.81	9.21	8.17	7.79	7.58	7.39	8.18	10.08	9.57	8.4
5	8.23	8.96	8.42	8.38	9.42	10.39	11.14	9.36	10.36	9.4
6	8.76	9.18	7.81	8.72	8.48	9.92	10.74	11.06	10.03	9.4
7	8.44	8.87	6.78	6.93	7.62	9.20	11.11	10.93	9.40	8.8
8	8.73	8.78	6.76	7.06	6.96	8.58	10.13	10.12	10.36	8.7
9	8.55	8.15	7.69	8.51	8.96	9.26	10.27	11.90	10.94	9.4
10	7.95	6.30	6.69	5.78	7.29	7.29	8.02	8.56	9.08	7.4

* The hemoglobin figures are averages of the values for each of the three sows on the individual rations.

We were not surprised to find this picture with the pigs farrowed from this sow because she had received only corn and milk which are low in iron and the low intake of this element may have caused low iron reserves in the young. However, we were surprised to find that every litter farrowed by these sows, without regard to the iron and copper addition to the basal ration, exhibited identical blood changes. Chart 1 gives a curve drawn from the average of the hemoglobin values of all the pigs which did not receive any additions after birth. The blood changes are identical with those described by McGowan and Crichton and by Doyle and coworkers. The other symptoms observed were also so similar to those described by these workers that it is unnecessary to discuss them further.

It is very important to note that pigs farrowed from sows receiving large additions of iron and copper develop anemia just as rapidly as the pigs farrowed from the sows on the basal ration. For the purpose of comparison we also present in Chart 1 the curves of the hemoglobin changes in two pigs from the litter farrowed by Sow No. 331 in Group 9, which had received daily additions of 50 mg. of iron and 50 mg. of copper during the entire gestation period. The slope of these curves is just as great as that of the upper curves shown in Chart 1 representing the pigs from the sow on the basal ration.

The facts demonstrate that the rate of anemia development in young suckling pigs is not affected by liberal additions of iron and copper to the ration of the sow. We can conclude therefore that the reserve supply of iron and copper in the pig at birth cannot be increased by feeding the sow these salts. The analysis of the livers taken from some of the pigs at birth gives results which agree with this conclusion. The livers were removed from the pigs directly after birth, dried and analyzed for iron and copper. A few of the results for pigs farrowed by sows fed the basal ration and sows fed this ration plus iron and copper additions are given in Table II. The iron content of the livers from the pigs farrowed by sows receiving 50 mg. iron daily was no higher than the iron content of the livers from the pigs farrowed by sows on the basal ration. The average of the figures for the copper content of the livers from sows receiving high levels of this element is slightly higher than the figures for the livers from the pigs farrowed by sows on the basal ration. However, the figures are not strikingly different, and more data are required before one could conclude that the copper content of the livers of young pigs at birth is influenced by the copper content of the sow's ration. The inability to definitely increase the reserve of blood forming elements in the pig at birth substantiates the findings in this laboratory of Lindow, Peterson, and Steenbock

(7) who showed that feeding large amounts of copper to rats during the gestation period did not delay the development of anemia in the young and did not increase the copper content of the young at birth. We can also conclude that the iron and copper content of the sow's milk is not influenced by the ingestion of these salts. This conclusion is in accord with our recent work (3, 8) which demonstrated that the iron and copper content of cow's milk and goat's milk cannot be increased by feeding iron and copper salts in the rations of these animals.

TABLE II

THE EFFECT OF THE SOW'S RATION ON THE IRON AND COPPER CONTENT OF THE LIVER OF THE PIGS AT BIRTH

Pig No.	Farrowed by sow in Group	Daily additions to the sow's ration		Iron content of dried liver per cent	Copper content of dried liver per cent
		Iron	Copper		
2	I	none	none	0.1520	0.0163
11	I	none	none	0.1160	0.0183
R. 1	V	none	2 mg.	0.0895	0.0254
R. 2	V	none	2 mg.	0.0946	0.0294
R. 3	V	none	2 mg.	0.1270	0.0200
42	VIII	50 mg.	20 mg.	0.0974	0.0254
43	VIII	50 mg.	20 mg.	0.2020	0.0305
45	VIII	50 mg.	20 mg.	0.1740	0.0320
B. 1	IX	50 mg.	50 mg.	0.1280	0.0341
B. 2	IX	50 mg.	50 mg.	0.0860	0.0306
B. 3	IX	50 mg.	50 mg.	0.0904	0.0320

We felt that a study of the effect of the ration on the rate of anemia development in the young was of such practical importance that the study should not be confined to restricted rations supplemented with iron and copper but should also include more practical rations. Therefore nine additional sows were selected from the general herd shortly after they were bred and divided into three lots of three sows each. Lot I was placed on the regular winter ration used by the Swine Department, consisting of yellow corn 35 parts, oats 29.5 parts, wheat middlings 20 parts, tankage 5 parts, oil meal 5 parts, chopped alfalfa 5 parts and salt 0.5 parts. Lot II received the basal ration of milk and corn used for the other series of sows and Lot III the same basal ration plus 100 mg. of Fe and 20 mg. of Cu per pig daily. These sows also received cod liver oil for protection against rickets.

When these sows farrowed, the hemoglobin of the young was followed

to note any difference in the rate of anemia development. The amount of hemoglobin in the pigs at birth was normal as we had found with all the other pigs. The average hemoglobin at birth for the pigs in the three lots was as follows: Lot I, 7.77 gm.; Lot II, 7.91 gm.; Lot III, 7.42 gm. As the pigs grew they all developed anemia regardless of the ration fed to the sow. The curves given in Chart 2 show that pigs farrowed from a sow fed a practical ration come down with anemia just as rapidly as the pigs from the sows fed the basal ration.

Treatment of Anemia in the Suckling Stage

Since the reinforcement of the sow's ration with iron and copper does not protect the young pig, the problem centers around the direct feeding of these elements to the suckling pig. Solutions of $Fe_2(SO_4)_3$ were prepared so that 1 cc. contained 25 mg. of Fe and solutions of copper sulfate were made so that 1 cc. contained 5 mg. of Cu. The ferric sulfate used contained small amounts of copper as an impurity. Dosages of 1 cc. of the solutions were then fed to the pigs with the aid of a pipette. Some of the pigs were given the iron alone, others were fed both the iron and the copper. The hemoglobin curves for four pigs from Sow 406 which are litter mates of pigs 109 and 110 whose hemoglobin changes are shown in Chart 2 are given in Chart 3. Pigs 112 and 113 received 25 mg. of Fe per pig daily and pigs 114 and 115 received 25 mg. of Fe and 5 mg. of Cu daily. Chart 4 similarly shows the hemoglobin changes in four pigs from Sow 306 which are litter mates of pigs 101 and 102 whose curves were shown in Chart 2. Pigs 103 and 104 were fed iron alone and pigs 105 and 106 were given both iron and copper. When the curves presented in these charts are compared with those in Chart 1 for the pigs restricted to sow's milk only, the effectiveness of the iron and copper treatment is definitely established. The response obtained by either iron alone or both iron and copper was exceptionally rapid, the hemoglobin reaching the normal level in two weeks and remaining at that value as long as the experiments were continued.

The iron and copper feeding to the suckling pigs not only stimulated hemoglobin synthesis but also increased the amounts of these elements in the livers of the young pigs. In Table III are given the quantities of iron and copper present in the livers taken from the pigs farrowed by Sow No. 306 when the young were 51 days old. Two pigs in this litter had received only sow's milk during this period, two had received 25 mg. of iron and two had received 25 mg. of iron and 5 mg. of copper during the last 45 days of the period. The iron content of the livers from pigs 101 and 102 had decreased to about 1/10 the amount normally present in the

liver at birth. Similarly the copper content decreased to about 1/5 of the amount found in the livers at birth. The iron content of the livers from the four pigs which received the iron additions for 45 days had increased to three times the amount in the livers from anemic pigs. The copper content of the livers from the two pigs fed iron alone was not increased, but the livers from the two pigs fed both iron and copper contained about three times the amount of copper found in the livers of the anemic pigs. However, the figures for the iron and copper content did not reach the amounts found in the liver at birth. It is important to note that the hemoglobin regeneration was rapid in the pigs receiving the iron alone, even though the copper content of the livers was not increased.

TABLE III

THE EFFECT OF IRON AND COPPER FEEDING TO SUCKLING PIGS ON THE QUANTITY OF THESE ELEMENTS IN THE LIVER

Pig No.	Pig's Ration	Iron in dried liver per cent	Copper in dried liver per cent
101	Milk alone	0.0128	0.00373
102	Milk alone	0.0100	0.00450
103	Milk plus iron	0.0330	0.00431
104	Milk plus iron	0.0314	0.00650
105	Milk plus iron and copper	0.0386	0.01300
106	Milk plus iron and copper	0.0296	0.00880

Further proof of the effectiveness of iron feeding is shown in Table IV. Hemoglobin values for the entire litter from Sow No. 331 are tabu-

TABLE IV

THE EFFECT OF IRON AND COPPER FEEDING ON THE HEMOGLOBIN CONTENT OF THE BLOOD OF SUCKLING PIGS. RECORD OF SOW NO. 331 WHICH FARROWED 7 PIGS MAY 6, 1929

Grams Hemoglobin per 100 cc. Blood

No. of Pig	Age in Days									
	1	7	14	15	23	30	38	46	53	60
42	7.94	4.30	2.75	—	3.57	dead				
43	8.28	5.47	3.77	—	3.48	3.55	3.15	2.96	3.53	2.71
44	5.69	3.25	2.12	Iron added	6.37	7.08	8.79	9.66	10.73	10.36
45	8.52	5.69	3.08	Iron added	6.74	8.40	9.35	8.79	11.82	9.21
46	8.65	5.37	3.38	Iron and copper added	6.45	7.62	8.40	9.84	10.94	9.66
47	8.92	4.96	2.92	Iron and copper added	6.04	7.83	8.52	9.48	11.82	10.94

lated, which show the condition of the blood stream in two pigs allowed only the sow's milk, in two pigs receiving iron additions, and in two pigs getting both iron and copper supplements. Again we see that very rapid improvement takes place in the blood stream, whether the iron is fed alone or the iron is supplemented with copper. The records presented in the table and the charts are typical of the improvement noted in all the pigs which received the iron treatment.

These results are in accord with those obtained by McGowan and Crichton (2) when they fed ferric oxide. These workers fed large quantities of ferric oxide to the sow and found that the anemia in the young was prevented by this treatment. The young pigs were undoubtedly benefited by the consumption of some of the iron in the feces. The iron in the ferric oxide must have been made more available during its passage through the digestive tract of the sow, because we have found that feeding ferric oxide directly to the young ameliorates anemia very slowly. In Table V

TABLE V

THE EFFECT OF FERRIC OXIDE FEEDING ON THE HEMOGLOBIN CONTENT OF THE BLOOD OF SUCKLING PIGS. RECORD OF SOW NO. 382 WHICH FARROWED 6 PIGS MAY 15, 1929

Grams Hemoglobin Per 100 cc. Blood

No. of Pig	Age in Days							
	1	10	19	27*	37	42	49	56
140	6.60	3.98	5.42	4.53	5.86	3.24	3.48	3.79
141	9.85	5.98	6.04	4.92	3.40	5.52	6.99	7.08
142	7.08	4.76	5.52	5.08	4.27	4.88	5.98	4.76
143	7.08	3.66	4.23	4.53	3.98	5.47	5.80	5.52
144	6.24	3.48	2.93	2.76	3.57	5.47	4.06	4.23
145	6.17	5.13	dead					

* Iron additions equivalent to 125 mg. Fe_2O_3 per pig started 27th day.

are given the figures which show the blood changes in anemic pigs when they were fed daily 125 mg. of Fe_2O_3 by capsule. These results show that the best means of preventing the anemia, or curing it after such a condition exists, is the feeding of a soluble iron salt directly to the young pig.

Work with Pigs Fed Cow's Milk

In all the work with the suckling pigs care was taken that the pigs were restricted entirely to sow's milk, *i.e.*, access of the young pig to the sow's ration was prevented as much as possible. The sows were removed to other pens when they were fed, thereby eliminating the presence of

food in the pens where the young pigs were kept. Even when these precautions were taken the pigs had access to the sow's feces. In order to eliminate this difficulty and to determine if iron and copper additions would bring about hemoglobin regeneration under more restricted conditions, some of the pigs were taken from the sow when 3 weeks old and fed cow's milk. Incidentally this procedure allowed us to determine what effect cow's milk has on the rate of anemia development.

In Chart 5 we present the hemoglobin values for three pigs; one on cow's milk alone, one on cow's milk plus an iron addition, and one on cow's milk plus both iron and copper additions. The iron used for these pigs was a purified $FeCl_3$ free from copper. We wished to determine if iron alone would prevent the anemia in the conditions under which these pigs were fed, or if it was necessary to add both iron and copper. It is seen from the chart that a pig becomes just as anemic when maintained on cow's milk as when allowed the sow's milk. The pig fed the pure $FeCl_3$ made almost as rapid recovery as the one fed both iron and copper. There may be two reasons for this fact. First, the iron reserves in the young pig may be depleted first and as soon as this element is supplied, hemoglobin synthesis takes place without the additions of copper. Second, the pigs were not placed under as restricted conditions as were the smaller animals used in our earlier experimental work, and were probably able to acquire small amounts of copper.

From a practical point of view, the feeding of iron is the important preventative of anemia in suckling pigs. However, it is entirely possible that the importance of copper can be demonstrated by keeping the pigs under very restricted conditions and allowing them to remain anemic for some time to insure a heavy drain on the copper reserves.

These pigs were kept indoors with no access to sunlight or other outside factors; still both the pigs fed the iron and the one fed iron and copper grew normally and were normal in every way when the experiment was discontinued. This shows that iron together with copper present in the reserves of the animals or acquired from the refuse will correct all the hemoglobin-forming deficiencies in sow's milk and that outside factors are not necessary for the prevention of anemia in suckling pigs. Further proof of this fact is given by the following work. Nine pigs were taken from the sow when about three weeks old and placed on cow's milk. When all the pigs were anemic, two were left on the basal ration of cow's milk; two were fed the basal ration plus 25 mg. of Fe as pure $FeCl_3$ daily; one was fed 25 mg. of Fe daily as pure $FeCl_3$ and 5 mg. of Cu as $CuSO_4$; two were placed on the basal ration and exposed ten minutes daily to a

quartz mercury vapor lamp; and two were kept on the basal ration and exposed thirty minutes daily to sunlight. The results of this work are given in Table VI. The response to the iron feeding is identical with all other pigs given iron, but it is readily seen that the ultraviolet light and sunlight are not effective in promoting hemoglobin synthesis.

TABLE VI

THE EFFECT OF DIFFERENT TREATMENTS ON THE ANEMIA IN PIGS RESTRICTED TO COW'S MILK

Grams Hemoglobin Per 100 cc. Blood

No. of Pig	Days After Treatment was Started				
	0	Treatment	8	15	22
432	5.42	Basal	5.37	5.32	4.17
434	4.92	Basal	5.37	5.04	6.11
435	4.43	Iron	6.31	6.67	7.62
433	5.98	Iron	8.28	8.34	10.01
430	4.76	Iron plus copper	8.28	10.01	11.54
1435	3.89	Ultra violet light	3.66	3.29	2.98
439	4.68	Ultra violet light	4.15	4.30	4.37
437	3.79	Sunlight	2.64	2.25	2.00
438	3.22	Sunlight	2.37	2.00	2.00

DISCUSSION

The demonstration of the presence of anemia in practically 100 per cent of the pigs studied may raise the question of why still greater losses are not encountered in pig raising. It is well to remember that all the pigs used in this study were restricted in pens with practically no access to other food than the sow's milk. When pigs are farrowed out of doors they start to root in the soil and consume green grass and soil material at a very early period in life. Apparently, in most cases, enough iron and copper are consumed to prevent severe anemia. When the pigs are kept indoors they become very anemic in three weeks time, if they do not consume additional food. In some cases we have noted gradual regeneration of the blood stream when the pigs consumed only small amounts of the sow's ration. If large amounts were consumed and the ration were richer in iron, the regeneration would undoubtedly have been much more rapid. However, in many cases the pigs succumb to anemia before they are old enough to hunt their own food.

The simplest way to insure against anemia is to allow the young pig sufficient iron. Just how the iron is supplied, whether by the direct ad-

ministration of iron or by the reinforcement of easily accessible food, apparently makes little difference. The solution of the entire problem lies in supplying the pig with iron during the period when milk constitutes the sole ration.

Conclusions

Sows restricted, shortly after weaning, to a yellow-corn-skim-milk diet and to floored pens maintain a normal hemoglobin level during the entire growing period. The addition of varying amounts of iron and copper to the ration does not increase the quantity of hemoglobin in the blood.

The pigs farrowed by these sows invariably develop a severe anemia in 3 to 4 weeks. The hemoglobin falls from a normal of 8 gm. per 100 cc. of blood at birth to 3 to 4 gm. per 100 cc. in a period of a few weeks. *The feeding of considerable amounts of iron and copper to the sow does not delay the development of anemia.*

Pigs farrowed by sows fed a practical winter ration developed anemia as rapidly as those from sows fed the basal ration of milk and corn.

The anemia in the suckling pigs is rapidly cured by the administration of iron and copper salts. The feeding of iron alone stimulates hemoglobin synthesis as well as when the iron is supplemented with copper.

Suckling pigs changed to a diet of cow's milk continue to develop anemia as rapidly as when allowed to remain with the sow. Under the experimental conditions, which we could establish with these pigs, the addition of pure $FeCl_3$, copper free, to the cow's milk, cures the anemia and maintains the pig in normal condition. The exposure of pigs on a cow's milk diet to ulatraviolet light and sunlight does not stimulate hemoglobin synthesis.

The severe anemia prevalent in suckling pigs kept indoors can readily be cured or prevented by administration of soluble iron salts.

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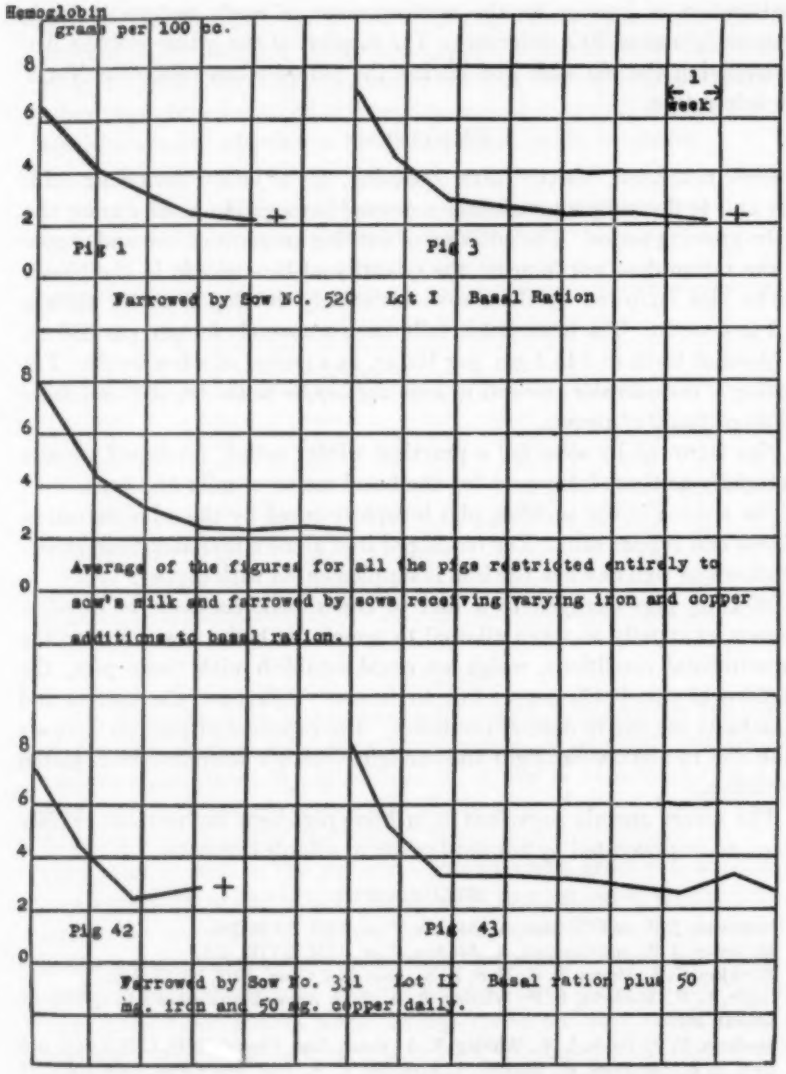
Hemoglobin
grams per 100 cc.
8
6
4
2
0
1 week
Pig 1
Pig 3
Farrowed by Sow No. 520 Lot I Basal Ration
Average of the figures for all the pigs restricted entirely to sow's milk and farrowed by sows receiving varying iron and copper additions to basal ration.
Pig 42
Pig 43
Farrowed by Sow No. 311 Lot II Basal ration plus 50 mg. iron and 50 mg. copper daily.

CHART 1.—In this chart are presented the hemoglobin curves for pigs restricted entirely to sow's milk and farrowed by sows receiving different iron and copper additions to a basal ration of yellow corn and skim milk. Hemoglobin determinations were made at weekly periods. The rate of anemia development in suckling pigs is not affected by the iron and copper intake of the sow. The cross at the end of the curve denotes death.

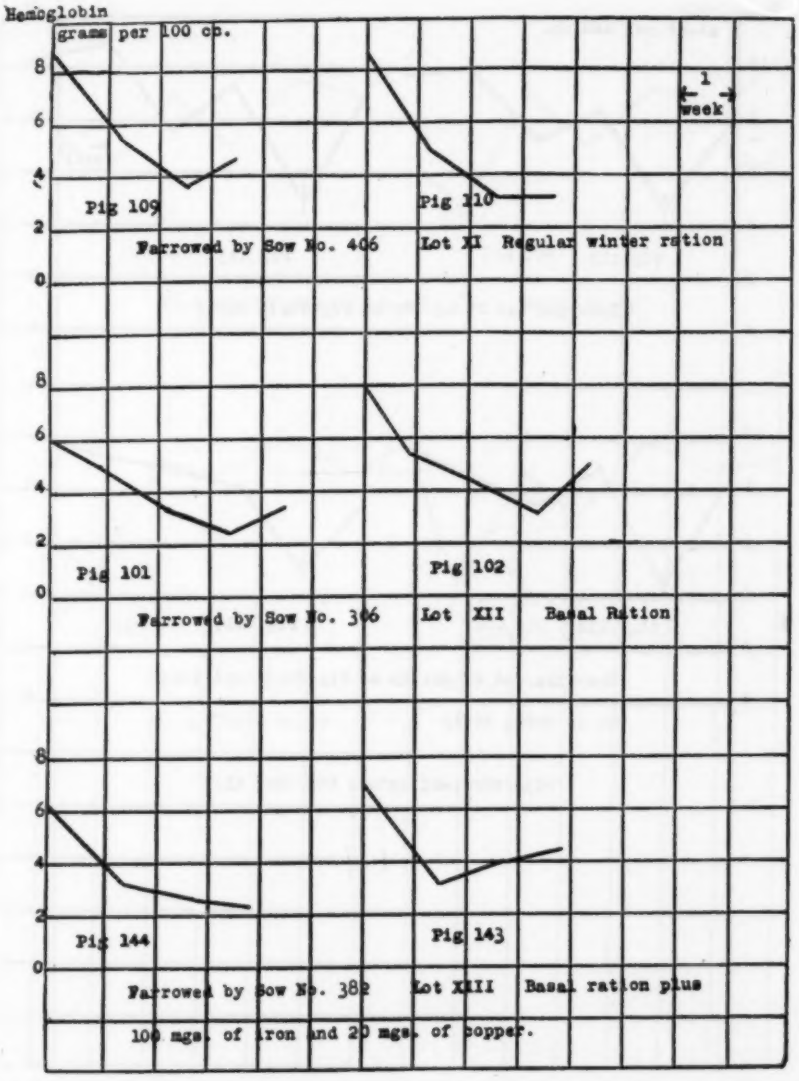

CHART 2.—The curves in this chart show the decrease in the hemoglobin content of the blood of pigs farrowed by sows on different rations when restricted entirely to sow's milk. The diet of the sow has no effect on the rate at which anemia develops in the young pigs.

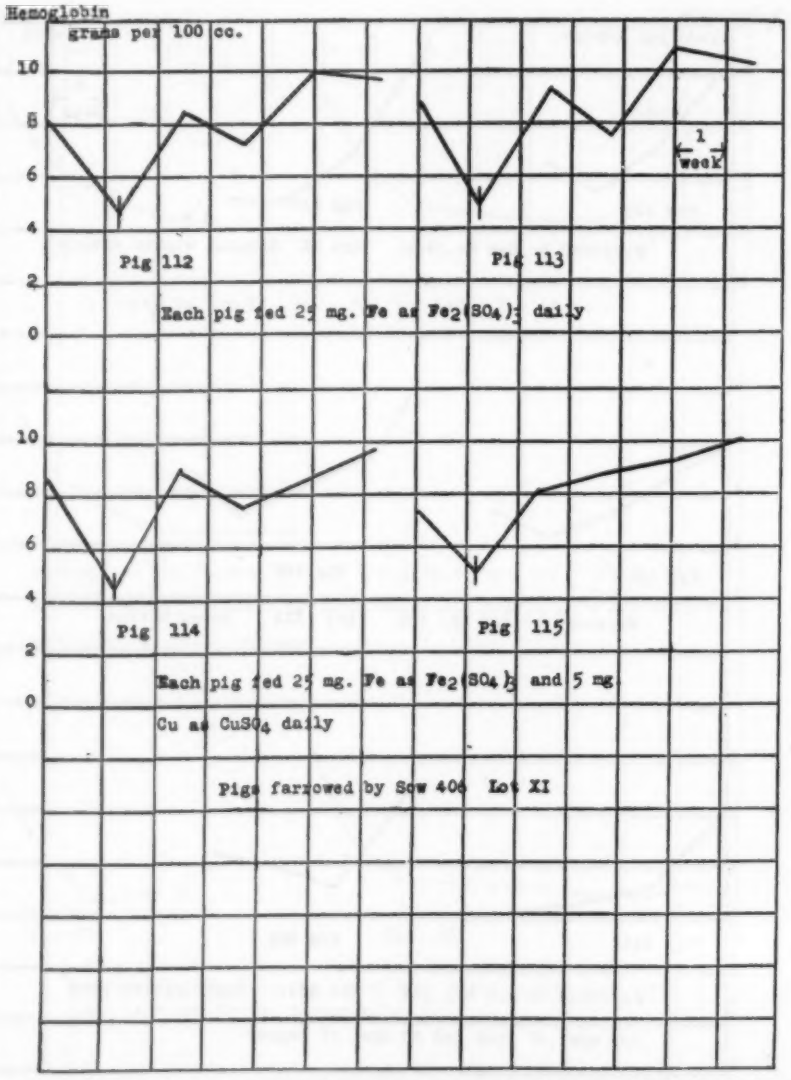

CHART 3.—The above curves show the rapid improvement in the hemoglobin of the blood of pigs when iron alone or iron plus copper is fed to the young pigs restricted entirely to sow's milk. The line across the hemoglobin curve indicates the point at which the additions to the diet were made.

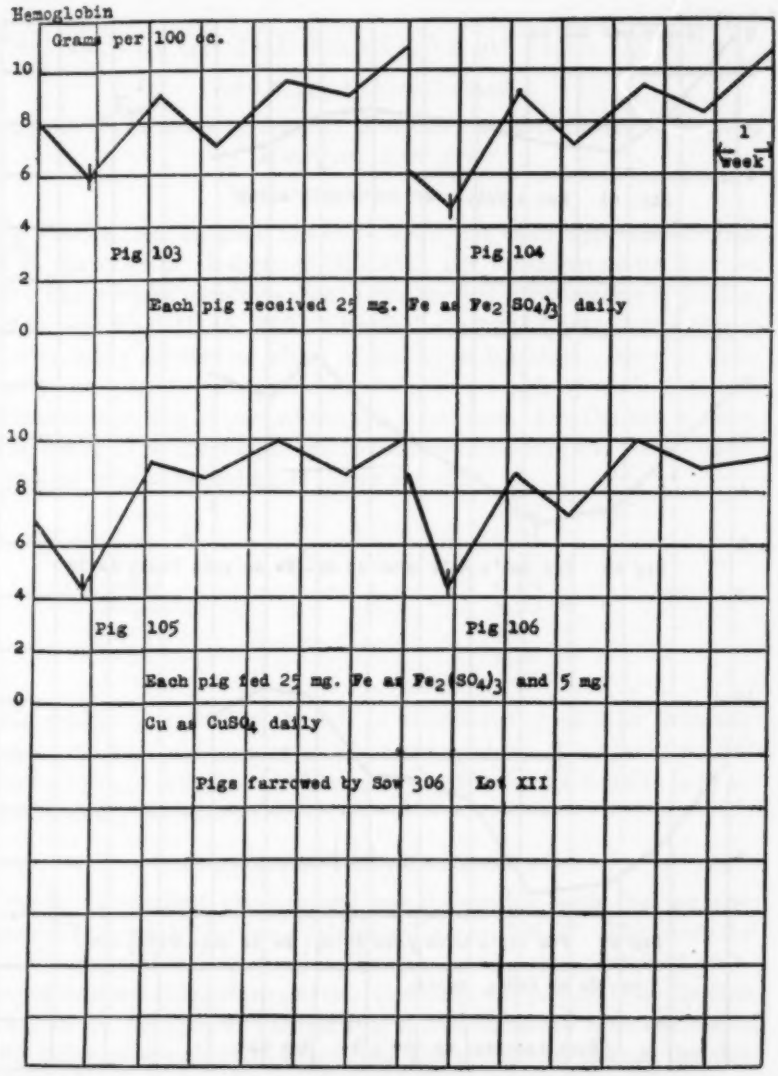

CHART 4.—Further curves are given in this chart showing the effect of iron and copper administrations to young pigs restricted entirely to sow's milk. The line across the hemoglobin curve indicates the point at which the additions to the ration were made.

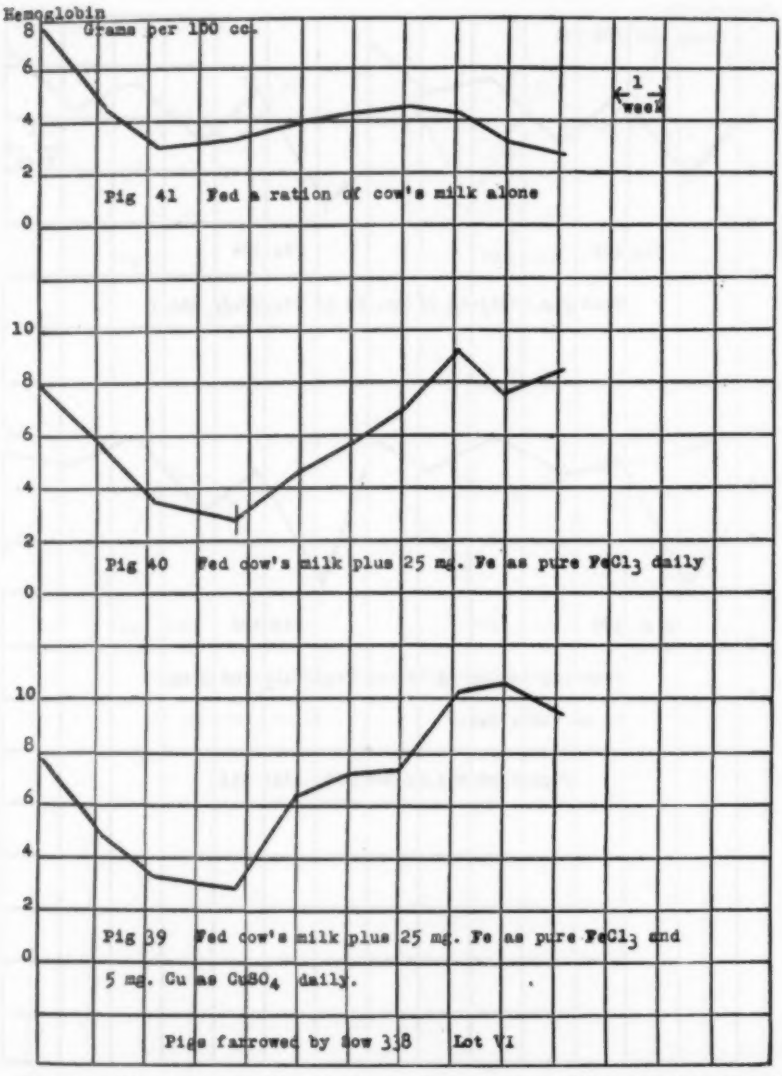

CHART 5.—The above curves show the effect of iron and copper additions to pigs restricted to a ration of cow's milk. The hemoglobin of the blood is maintained at a normal level whether pure $FeCl_3$ is fed alone or whether it is supplemented with copper. The line across the hemoglobin curve indicates the point at which the additions to the ration were made.

THE CAUSE OF THE LAXATIVE ACTION OF BRAN

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WHILE the laxative nature of bran has been appreciated since the days of Graham (1794–1851), the causation of its laxative action has received desultory study since the first investigation of Jordan, Patten, and Hart (1) in 1906. Chemical analysis of bran shows that it contains many substances which might act as laxatives. Some of these constituents are: 1.—Undigested carbohydrates such as crude fiber and pentosans occurring as part of the cell membrane. 2.—The ash of bran, which amounts to about 6 per cent of the unwashed bran and is distributed in part as follows according to Sherman: (2)

Calcium	0.120	per cent
Magnesium	0.511	" "
Potassium	1.217	" "
Sodium	0.154	" "
Phosphorous	1.215	" "
Chloride	0.090	" "
Sulphur	0.247	" "
Iron	0.0078	" "

It can readily be seen that there is an abundance of minerals definitely known to be laxative (as *e.g.* magnesium and sulphur). 3.—A compound of phosphorous and carbohydrate known as phytin to which has also been ascribed potency as a laxative.

Summary of Previous Reports

One of the earliest experimental reports dealing with the laxative nature of bran was by Jordan, Patten and Hart in 1906 (1). They were the first to suggest that bran owed its laxative nature to a contained phosphorous compound known as phytin. Certainly this seemed to be the case with cows for, they write: "In our attempts at transition from one ration to the other we soon learned that it was not possible to change the cow suddenly from a ration rich in phytin to one low in this compound and keep the animal in normal health. Such a change caused her to become seriously constipated and recourse to a purgative was necessary." They secured a ration rich in phytin by using unwashed bran and one low or absent by using wheat bran which had been allowed to soak over night

in water slightly acid to litmus. They further studied the metabolism of the phosphorous contained in phytin and concluded that after being reduced to inorganic phosphorous combinations it was excreted chiefly in the feces and slightly in the urine.

Their analyses of both the unwashed and washed bran showed that the washing had removed the greater portion of the magnesium and potassium compounds and but very little of the calcium compounds. They give this table:

INORGANIC CONSTITUENTS OF BRAN BEFORE AND AFTER LEACHING

	P	CaO	MgO	K_2O	Totals
	%	%	%	%	%
Whole bran.....	1.420	0.182	0.894	1.580	4.076
Washed bran.....	0.145	0.380	0.162	0.084	0.971

It is thus seen that bran washed in acidified water not only contains little phytin but also has a markedly lower mineral content, especially of such laxative mineral compounds as the magnesium salts.

They suggested that the true source of the laxative action of bran lay therefore in its contained phytin rather than in the fiber.

Shortly after the previous publication, Mendel and Underhill (3) reported on the physiological action of phytin in the dog and the rabbit. They found that the compound was readily absorbed and had no characteristic effects upon the health of the animals. However, the phosphorous of the phytin in both the dog and the rabbit was almost entirely eliminated through the kidneys rather than the feces—quite the opposite of the cow but similar to the situation in man. They thought that this mode of excretion of the phytin-phosphorous might be intimately related to its laxative action, for in the dog and rabbit purgation could not be constantly provoked. If the phytin-phosphorous had been excreted by the intestine, then, perhaps, it would have formed inorganic phosphorous compounds (*e.g.*, sodium phosphate) which might then have acted as a laxative.

In 1909, Hart, McCollum, and Humphrey (4) studied the question further, using the cow as an experimental animal. They confirmed the earlier work showing the laxative effect of phytin in the cow, furthermore maintaining that in this animal crude fiber had but little effect in relieving constipation. They also noted that "when the washed bran ration was supplemented with a quantity of magnesium and potassium as chlorides and sulphates, equivalent to these bases removed in the washing process, a sudden change from the whole bran to the washed bran ration could be made without in any way modifying the character of the feces which

remained normal." This again tended to support the hypothesis of Mendel and Underhill that phytin owed its cathartic nature to laxative mineral compounds that were formed in its katabolism and were excreted through the gut.

The latest report dealing with the subject is that of Williams (5). Dogs were used as the subjects of the experiments. They were fed a synthetic control diet, made according to Cowgill's method (6), to which were added varying amounts of crude fiber or other test objects. The number of defecations and the total weight of dried feces in each metabolism period were used as the criteria of laxation. The washed bran used in these experiments was prepared by washing it with cold water until it gave negative tests with iodine. The bran was then dried and used. It should be pointed out that washing bran with water alone will not remove all of the phytin. Averill and King (7) have shown that extraction with water alone causes wheat bran to lose about half of its phytin content but not much more. The washed bran used by Williams had a content of crude fiber of 17.24 per cent; of ash 3.12 per cent; and of pentosans, 36.94 per cent. He found that washed bran possessed a markedly laxative effect and that most of this laxative effect could be produced by the crude fiber of the bran.

The chemical nature of phytin has been most thoroughly investigated by R. J. Anderson (8). He concludes that the phytin of bran is probably inosite hexaphosphate, $C_6H_{18}O_{24}P_6$. Naturally occurring phytin is mainly a mixed calcium, magnesium, and potassium salt of inosite hexaphosphate.

The phytin content of food stuffs has been studied by Averill and King (*loc. cit.*) and by E. Arbenz (9). Both find the phytin content of wheat bran to be 4.5 per cent.

Experimental Procedure

To investigate this subject more fully a series of experiments was performed using the white rat. Special metabolism cages were constructed in such fashion that the feces were separated from the rat as soon as voided. (See Figs. 1 and 2). Non-scattering diet cups were used. The feces were collected every 24 hours and were placed in a drying oven (kept at 70° C). It was not feasible to measure the output of wet feces since considerable drying occurred at room temperature. The weight of the dry feces for the total experimental period was then determined after the feces had come to constant weight in the drying oven. The beginning and end of each feeding period was determined by carmine which usually took from 18 to 24 hours for appearance.

FIG. 1.—Metabolism cage for collection of rat feces, assembled.

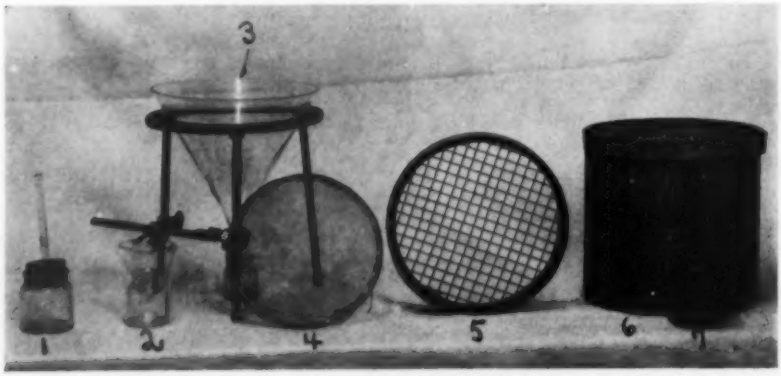

FIG. 2.—Component parts of metabolism cage for collection of rat feces.
Description of parts at bottom of page opposite.

In the first experiment, a group of rats was fed a basic diet which contained no bran, fiber, phytin, or bran-ash. In subsequent experiments varying amounts of these substances were added to the basic diet to determine the effect on laxation.

Two basic diets were used differing only in the source of their protein —in the one casein, in the other dried beef powder. It was found that the consumption of the casein diet was not sufficient in many cases to maintain the weight of the adult rats used but the beef diet was satisfactory in that regard. The basic casein and beef diets consisted of the following:

Casein	18.0	Dried Beef Powder	15.0
Starch	53.0	Starch	61.0
Salt mixture (Osborne & Mendel)	4.0	Salt mixture	4.0
Lard	20.0	Lard	17.0
Cod liver oil	3.0	Cod liver oil	3.0
Yeast concentrate[1]	2.0		
	100.0	plus daily vitamin B tablet	100.0

The results of feeding these diets to a group of 6 rats are summarized in Table I.

Table I shows that the 6 rats on the basal casein diet excreted an average of 7 stools daily with a total dry weight of 258 mg. and individual weights of 38 mg. The 2 rats on the basal beef diet excreted an average of 3.8 stools daily with a total dry weight of 220 mg. and an individual weight of 59 mg.

Three criteria of laxation may be used with the rat: 1. The daily number of stools, 2. The dry weight of each stool, 3. The total dry weight of stool produced daily. Hence one may observe a double action of laxatives; 1.—in increasing the number of stools, 2.—in increasing the weight of each stool. Either of these independently may increase the total daily weight of the feces and this separate effect was observed.

1. Three ounce wide-mouthed bottle with drinking tube six inches long and 5/16 in. in diameter.
2. Non-scattering food cup consisting of,
   a. 200 c.c. tall beaker, 3½ in.×2½ in.
   b. wide-mouthed 2 oz. bottle with mouth 1 3/16 in. wide.
3. Glass funnel, 8 in. dia.
4. Wire screen, 6¾ in. dia., 1/16 in. mesh.
5. Wire screen, 8¾ in. dia., ½ in. mesh.
6. Galvanized iron cage, 8¼ in. dia.
7. Cup holder.

[1] "Vegex."

It will be noted that half of the rats on the basal casein diets lost considerable amounts of weight probably due to their failure to ingest sufficient amount of the diet. It is possible that their stool production was somewhat subnormal but not sufficiently so to invalidate their use

TABLE I

THE EFFECT ON FECAL PRODUCTION OF BASIC CASEIN AND BEEF DIETS

Rat No.	No. days on diet	Change in rat wt. grams	Diet* grams	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Type of stool	Type of diet
1.	9	−20	4.7	238	6.0	40	small	casein
2.	9	−40	3.2	203	7.6	27	"	"
3.	9	− 3	6.55	245	5.6	44	"	"
4.	9	−20	5.0	264	8.7	30	"	"
5.	9	− 2	6.9	300	8.9	34	"	"
6.	9	+ 1	6.35	300	5.5	55	"	"
Av. of casein series		−14	5.78	258	7.0	38	small	
4.	5	+ 4	5.2	216	4.4	49	"	beef
5.	5	+ 6	4.4	224	3.2	70	"	"
Av. of beef series		+ 5	4.8	220	3.8	59	small	

* Daily averages.

in determining the average. If we arrange the 6 casein rats according to the daily grams of diet ingested, the following series may be constructed.

Rat no.	Daily diet in grams.	Av. Total daily stool in mg.	Daily no. stools	Wt. each stool in mg.
2.....	3.2	203	7.6	27
1.....	4.7	238	6.0	40
4.....	5.0	264	8.7	30
6.....	6.35	300	5.5	55
3.....	6.55	245	5.6	44
5.....	6.9	300	8.9	34

In general the rats with higher food intake have a somewhat larger weight of daily stools but there is no constant effect either upon the number of stools or upon the weight of each stool.

After determining the stool production of rats upon the basal fiber-free diet, they were fed diets containing unwashed bran, phytin-free bran, bran fiber, bran ash, and phytin with the purpose of ascertaining to what constituent bran owed its laxative nature. The unwashed bran had a crude fiber content of 9 per cent as determined by the method of the Association of Official Agricultural Chemists (10). Its ash content was

6 per cent. Its phytin content was not determined, but analyses by Averill and King and by Arbenz (*loc. cit. supra*) show that the phytin percentage of bran is 4.5.

Two rats were fed a diet containing 2.5 per cent of bran. The diet consisted of the following:

Unwashed bran	2.5
Casein	18.0
Starch	50.5
Salt mixture (O. & M.)	4.0
Cod liver oil	3.0
Yeast concentrate	2.0
Lard	20.0
	100.0

Table II is a summary of the results.

TABLE II

THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 2.5 PER CENT BRAN

Rat No.	No. days on diet	Change in rat wt. grams	Diet* grams	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	Dry* stool mg.	No. of stools*	Av. wt. of ea. stool mg.	Type of stool
1.	9	−10	6.8	170	15	7.5	10.2	329	7.5	44	small
2.	6	− 2	6.5	162	14.6	7.3	9.7	269	7.0	38	"
Averages			6.65	166	14.8	7.4	9.9	299	7.25	41	"

* Daily averages.

It is thus seen that when rats eat bran at the average rate of 166 mg. daily there is little if any laxative effect. Both these rats weighed about 190 grams and one may therefore calculate that a daily ingestion of unwashed bran of 0.9 grams per kilogram of rat weight is without appreciable effect on laxation. On the other hand, the addition of the bran to the diet did increase its palatability to the rat for we find increased ingestion with slight gains in weight; upon the basal casein diet both these rats had lost weight markedly.

Unwashed bran was next fed to a group of 3 rats at a dietary level of 5 per cent. The diet consisted of the following:

Unwashed bran	5.0
Casein	18.0
Starch	48.0
Salt mixture (O. & M.)	4.0
Lard	20.0
Cod liver oil	3.0
Yeast concentrate	2.0
Total	100.0

The results of feeding the 5 per cent bran diet are summarized in Table III.

When bran is ingested at the rate of 300 mg. daily or over one begins to see its laxative effect. This effect is three-fold: 1.—There is an increased number of stools, 2.—there is an increased total weight of dried stools, and 3.—there is a slight increase in the size of each stool. This third result is better seen in the fresh stool than by considering the weight of the dry stool. The bran feces are apparently more moist than the normal feces and the increase in the weight of the individual stool would probably be more marked if one were to compare moist weights rather than dry weights. The increased moisture content of feces produced by feeding bran was also noted by Williams (5) in the dog.

It should be observed in connection with this feeding experiment that whereas the feeding of 0.9 gram of bran per kg. of rat was without laxative effect, the feeding of amounts above 1.42 gm. per kg. was definitely laxative. Both rats 5 and 6 (See Table III) ingested about 300 mg. of bran daily but rat 6 had a greater fecal output and ate 1.94 gm. of bran per kg. whereas rat 5 ate 1.42 gm. per kg. Rat 7 showed an enormous increase in all items, especially in daily number of stools, in total dried weight of stool, and in grams of bran per kilogram. The dry weight of the individual stool was but slightly affected.

To study the effects of bran at a high level of intake a diet was fed which contained 10 per cent of unwashed bran. This diet was composed of the following:

Unwashed bran	10.0
Casein	18.0
Starch	43.0
Salt mixture (O. & M.)	4.0
Lard	20.0
Cod liver oil	3.0
Yeast concentrate	2.0
Total	100.0

It was fed to a group of 6 rats and the results are tabulated in Table IV. This table has been arranged according to the level of daily ingestion of bran so that, for example, the first rat (No. 6) has the lowest bran ingestion and the last rat (No. 7) has the highest. All the rats did well on this diet; there was an average gain of weight for the group of 10 grams. The daily level of bran ingestion ranged from 560 mg. to 850 mg. The total daily weight of dried stool was quite high and ranged from 520 to 870 mg. Furthermore, with the exception of rat 1, the weight of dried stool in-

TABLE III

THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 5 PER CENT BRAN

Rat No.	No. days on diet	Change in rat wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	No. of stools*	Type	Dry stool* mg.	Wt. each* mg.	Wt. rat gm.	Bran per kg. gm.
5.	9	+ 4	5.9	295	26.5	13.3	17.4	7.8	med.	312	40	208	1.4
6.	9	+ 1	6.2	310	27.9	14.0	18.6	7.1	"	430	61	160	1.9
7.	6	+20	12.1	610	55.0	27.5	36.6	14.6	"	810	56	220	2.8
Averages			8.08	405	36.4	18.2	24.2	9.8	"	517	52		2.0

* Daily averages.

TABLE IV

THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 10 PER CENT BRAN

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. each mg.	Type	Bran per kg. gm.
6.	10	154	− 4	5.6	560	50.3	25.1	33.0	520	8.5	61	med.	3.3
1.	10	205	+ 6	6.6	660	59.0	29.5	39.6	870	9.6	91	large	3.2
5.	10	214	+ 8	6.7	670	60.3	30.1	40.2	560	7.8	72	med.	3.1
8.	10	247	− 3	8.2	820	74.0	37.0	49.2	665	14.7	45	"	3.3
4.	7	165	+22	8.3	830	74.5	37.4	49.8	760	14.8	51	small	5.0
7.	10	237	+10	8.5	850	76.5	38.2	50.9	753	13.3	57	med.	3.6
Averages				7.3	730	65.7	32.8	43.8	688	11.4	60		3.6

* Daily averages.

creased proportionally with the increase in dietary bran. The number of stools was markedly increased and the weight of the individual stool slightly increased. Bran, therefore, in causing laxation does so both by increasing the number of stools and the size of each stool. It cannot be said which is more affected since the wet weight of the stool is unknown. The bran ingestion calculated on the basis of kilogram of rat weight is fairly constant, ranging from 3.1 to 3.6 grams with the exception of rat 4 which ate such large amounts of diet that a gain in body weight of 22 grams resulted.

To bring out the effects of bran in a quantitative fashion, a table was constructed in which the percentage of bran in the diet was ignored but the actual milligrams ingested were progressively set down. In Table V one may therefore determine the probable stool production at a given level of bran ingestion. The averages of the basal casein diet are included for comparison. The laxative effect of bran begins when 300 mg. or more are ingested daily or at a level of about 2 grams per kg. of rat weight. The proportionality between bran ingested and fecal production is clearly shown. The two rats marked with an asterisk are the only exceptions to the general trend.

TABLE V

RELATION BETWEEN BRAN INGESTION AND FECAL PRODUCTION

Rat No.	Av. Daily bran mg.	Daily dry stool mg.	Av. Daily no. of stools	Bran per kg. gm.
Casein average	0	258	7.0	0.0
2	162	269	7.0	0.9
1	170	329	7.5	0.9
5	295	312	7.8	1.4
6	310	430	7.1	1.9
6	560	520	8.5	3.3
7*	610	810	14.6	2.8
1*	660	870	9.6	3.2
5	670	560	7.8	3.1
8	820	665	14.7	3.3
4	830	760	14.8	5.0
7	850	753	13.3	3.6

We may conclude that bran quantitatively increases fecal production. What element in the bran is responsible for the increase?

Reverting to Table IV, one notes that the group of animals in which bran was most laxative were ingesting from 50 to 60 mg. of bran crude

fiber daily, 25 to 33 mg. of phytin, and from 33 to 44 mg. of bran ash. All of these substances increased or decreased exactly as the bran intake. Were they responsible for its laxative effect? The following types of diets were fed to answer this question:

1. A diet containing bran which had been rendered phytin-free and low in ash content; 2. A basal casein or beef diet to which varying amounts of the crude fiber of bran were added; 3. A basal diet to which phytin was added; 4. A basal diet to which bran ash was added.

The Effect of Feeding Phytin-Free, Low Ash Bran

Bran which was free of phytin and very low in ash content was prepared as follows:

1. Unwashed bran was extracted in 2 per cent HCl for six hours. 2. The bran was washed thoroughly with distilled water. 3. The bran was dried in the oven at 50°C. to a constant weight. 4. It was then dried in room air for 24 hours.

This air-dried bran had an ash content of 0.4 per cent. Its crude fiber and pentosan content were not determined but these substances must have been increased in percentage at least by the amount of water-soluble materials that were lost, the soluble carbohydrates, phytin, and ash. Any laxative effect of phytin-free, low-ash bran would therefore owe its potency to something other than phytin or ash.

Table VI is a tabulation of the results of a diet containing 10 per cent of phytin-free bran prepared as indicated above. The diet consisted of the following:

Phytin-free bran	10.0
Casein	18.0
Starch	43.0
Salt mixture (O. & M.)	4.0
Lard	20.0
Cod liver oil	3.0
Yeast concentrate	2.0
Total	100.0

The table is arranged in order of increasing ingestion of bran. Several conclusions may be drawn with regard to the effect of ingesting the phytin-free diet. First, all the animals ate less of this diet than of the diet containing the unwashed bran and in fact ate so much less that they all lost weight. Evidently washing bran with 2 per cent HCl decreases its palatability. Secondly, there is a clear proportionality between the total weight of dry stool and the amount of bran ingested; rat 1 is the only

exception to the trend. Thirdly, a diet containing no phytin and far less bran ash than any previously fed proves to be markedly laxative. The average total weight of daily stools for the group is 674 mg. as compared with 688 for the group receiving 10 per cent unwashed bran. Moreover the stools are increased in size and number to the same degree.

One may state unequivocably that the phytin of bran plays no part in producing laxation in the rat. Similarly, the ash of bran is of little or no importance. The phytin-free bran does, however, contain a very high percentage of both crude fiber and pentosans since the extraction with dilute hydrochloric has removed most of the soluble substances. While the crude fiber was not estimated it may be calulated that the fiber content of the phytin-free bran was increased to at least 19 per cent. The extraction removed 4.5 per cent of contained phytin and 5.6 per cent of contained minerals. Therefore the crude fiber content of unwashed bran of 9 per cent must have been increased to at least 19 per cent. The pentosans were not determined. Williams (*loc. cit.*) has found that washed bran has a pentosan content of 36.94 per cent. Thus pentosans and crude fiber amounted to over 50 per cent of the phytin-free bran.

TABLE VI

THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 10 PER CENT OF PHYTIN-FREE BRAN

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. each mg.	Type	Bran per kg. gm.
6.	9	143	−18	4.0	400	80**	1.6	470	8.5	55	med.	2.8
4.	9	174	− 3	4.45	445	89	1.8	507	8.0	63	large	2.6
5.	9	208	−20	4.8	480	96	1.9	610	11.3	54	med.	2.2
1.	9	197	−21	5.8	580	116	2.3	818	7.8	105	large	2.9
7.	9	237	−10	7.35	735	147	2.9	759	13.3	57	large	3.1
8.	9	240	−10	7.55	755	151	3.0	881	17.0	52	med.	3.1
Averages			−14	5.65	565	113	2.3	674	11.0	61		2.8

* Daily Averages. ** Minimum Values.

If bran owes its laxative effect to the unabsorbed food residues arising from crude fiber and pentosan, then one would expect a strikingly laxative effect from crude fiber fed in amounts equivalent to the combined pentosans and crude fiber.

To test this deduction, a group of 7 rats was fed varying amounts of crude fiber incorporated in the basal beef diet. The results are exhibited in Table VII. The table is again arranged according to the average amount of fiber ingested daily.

TABLE VII

THE EFFECT OF CRUDE FIBER OF BRAN ON FECAL PRODUCTION

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Crude fiber* mg.	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Crude fiber kg. gm.
7	8	224	−16	45	275	5.6	49	0.20
8	8	224	−23	48	275	7.0	39	0.21
9	6	218	+12	76	529	12.0	44	0.35
14	6	161	+18	162	523	15.0	35	1.01
15	6	140	+14	180	610	17.0	35	1.29
11	3	170	+ 4	570	811	18.0	45	3.34

* Daily averages.

The crude fiber used in this experiment was obtained from bran by the following method:

1. Unwashed bran was extracted with ether overnight to remove the fat. 2. The ether was decanted and the bran dried. 3. The bran was boiled for 30 minutes with 1.25 per cent sulphuric acid solution in a large round-bottomed flask to which was attached a reflux condenser. 4. The acid mixture was strained through cheese cloth and washed with hot water until neutral to litmus. 5. The residue was returned to the flask and boiled for 30 minutes with 1.25 per cent sodium hydroxide solution. 6. The straining through cheese cloth and washing with water was repeated. 7. The residue was dried to constant weight at 90° C. This method of obtaining crude fiber is closely similar to the standard methods for determining crude fiber. It does not include pentosans and the value given before for the crude fiber of unwashed bran (9 per cent) does not include pentosans. Crude fiber is essentially cellulose and the pentosans are the hemicelluloses, which require a different method for determination.

Table VII shows that with increasing concentrations of crude fiber there is increasing laxation. Interestingly enough, the crude fiber of bran produces an increased number of stools which in the moist state re-

semble the bran feces but when dry show no increased individual weight above normal.

While it has been shown that the absence of phytin or of bran ash does not in any way lessen the effectiveness of bran as a laxative, the question arises whether these substances may not have some inherent laxative effect. This was investigated by feeding to a group of 5 rats, phytin and ash at concentrations even higher than are present in the 10 per cent unwashed bran diet. Referring to Table IV, it will be seen that in this diet the average ingestion of bran ash was 43.8 mg. daily and of phytin 32.8 mg. daily. The following diets were fed:

1.–2 per cent phytin, 2.–0.6 per cent bran ash, 3.–1.2 per cent bran ash. The composition of the first diet was as follows:

Phytin	2.0
Casein	18.0
Starch	53.0
Salt mixture	4.0
Lard	20.0
Cod liver oil	3.0
plus daily vitamin B tablet	100.0

The bran ash diets were composed as follows:

Bran ash	0.6	1.2
Dried powdered beef	15.0	15.0
Starch	60.4	59.8
Salt mixture	4.0	4.0
Lard	17.0	17.0
Cod liver oil	3.0	3.0
plus daily vitamin B tablet	100.0	100.0

The phytin was prepared from bran by the method of Clark (11): Two and one-half pounds of bran were extracted for five hours with 2.0 per cent hydrochloric acid at a room temperature of about 20°C. The extract was filtered through cheese cloth and allowed to settle overnight. The clear, supernatant liquid was siphoned off, heated to boiling to coagulate the protein, and allowed to cool. After settling, the clear amber-colored liquid was again siphoned off from the residue, and heated to boiling. Ammonia was added until the solution was just alkaline and the boiling continued for a short time. A large quantity of a flocculent precipitate separated out. This was filtered while the solution was still hot, and was washed with boiling water. This brown, sticky precipitate was extracted with two liters of 8.0 per cent acetic acid, and filtered from an insoluble residue. The amber-colored solution thus obtained was heated to boiling, and a large

amount of phytin separated out as a fine, white precipitate which redissolved on cooling except for a slight insoluble residue which was filtered off. The clear filtrate was diluted with an equal volume of water and heated to boiling, and again made alkaline with ammonia. A large, white precipitate settled out, which was again filtered while hot, and washed thoroughly with boiling water. This white precipitate was extracted with the smallest possible amount of 0.8 per cent acetic acid, and filtered from a slight insoluble residue. This acetic acid solution was heated to boiling, and the phytin separated out as a heavy white, powdery precipitate, which was filtered off while hot, leaving in the filtrate all inorganic phosphates which were soluble in the acetic acid solution. This precipitate was thoroughly washed with hot water, alcohol, and ether, and dried in the air at room temperature.

Bran ash was prepared by ashing large quantities of unwashed bran in large silica dishes over open burners. After the ashing was completed the residue was finely powdered and incorporated in the diet.

Table VIII summarizes the results with phytin and bran ash. Phytin is without laxative effect when ingested at the rate of 72 to 84 mg. daily. This is over twice the amount ingested by the animals in the 10 per cent unwashed bran diet. Bran ash is without effect when eaten at a level of 48 to 66 mg. daily. This is slightly higher than the average ingestion of the 10 per cent bran group. When the amount ingested is increased to about 100 mg. daily there is a slight laxative effect in one of the rats (No. 13) and none in the other (Rat 12).

TABLE VIII

THE EFFECT OF PHYTIN AND BRAN ASH UPON FECAL PRODUCTION

Type of diet	Rat No.	Days on diet	Change in rat wt. gm.	Diet* gm.	Phytin* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Type of stool
2% phytin	7	5	−16	3.6	72	0	220	5.0	43	small
2% "	8	5	− 4	4.2	94	0	302	7.6	40	"
0.6% ash	10	4	+10	7.8	0	48	232	7.8	30	"
0.6% "	11	6	+14	9.8	0	66	116	7.0	17	"
1.2% "	12	4	+12	7.8	0	94	251	7.7	32	"
1.2% "	13	6	+16	9.8	0	118	390	9.0	43	"

* Daily averages.

It is not surprising that bran ash in very high concentrations should have some laxative effects because of its contained magnesium and sulphur salts. However, the effect is small and not constant.

Summary and Conclusions

1. A method of studying the fecal output of the rat is described. In the rat:

2. Bran acts as a laxative in a quantitative fashion—increasing amounts of bran produce increasing weights of feces.

3. Phytin-free bran has lost none of the laxative qualities of bran.

4. The ash of bran and the phytin contained in bran are not laxative in the quantities in which they are contained in bran. Bran ash fed separately may be slightly laxative in high concentrations.

5. Bran owes its laxative action to the crude fiber and pentosans which it contains.

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Editorial Review

THE EMPTYING MECHANISM OF THE STOMACH*

THE first important contribution to the physiology of the alimentary tract made on this continent was Beaumont's (1833) remarkable study of the stomach of Alexis St. Martin. To commemorate this achievement a *fac simile* copy of Beaumont's book, containing also Osler's (1902) well-known address on Beaumont as an introduction, was presented to each member of the recent International Congress of Physiologists at Boston. Beaumont's book appeared in 1833 and one of the four periods covered by his observations began in August 1829 and continued until March 1831. This issue of the Journal therefore falls within the 100th anniversary of Beaumont's labors. It is for this reason that this discussion appropriately begins with a brief characterization of this first American contribution.

A very brief allusion to the circumstances which gave Beaumont, an obscure Army surgeon, his great opportunity must suffice; for every text book of physiology in the past hundred years has made some reference to the case. St. Martin, a youth of 18, was wounded by the accidental discharge, at very close range, of a shot gun loaded with duck shot. The charge entered his body posteriorly, traversed the lower lobe of the left lung, tore away a piece of the diaphragm, perforated the stomach, and passed out through the abdominal wall just below the costal margin and only two inches below the left nipple on a line from the nipple to the crest of the ilium on the same side. A large, lacerating wound was left. Beaumont, then stationed at Michilimackinac, where the accident occurred, reached the boy within 25 minutes, dressed his wound, and though despairing of his recovery, took him into his family later and personally nursed him back to health. Only after two years was the young man able to work. Beaumont began his observations in 1825, nearly three years after the accident, and took the subject with him to various army posts where he was stationed and kept up his experiments as best he could with a rather stupid and at times recalcitrant individual for the next eight years.

Osler, in the address to which reference has been made, quotes the following lines from Combe, a contemporary professor at Edinburgh, to indicate the value of Beaumont's work. "It would be difficult to point out any observer who excells him in devotion to truth and freedom from the trammels of theory or prejudice. He tells plainly what he saw and leaves every one to draw his own inferences, or where he lays down conclusions

* Revised from an address delivered before The Philadelphia County Medical Society, Phila., Pa., on November 26, 1929.

he does so with a degree of modesty and fairness of which few perhaps in his circumstances would have been capable."

To give an idea of the state of knowledge, or rather lack of knowledge, of digestion, at this time, Osler refers to Dunglison who, in his "Work on Human Physiology" (published the very year of Beaumont's book) after discussing all the old theories regarding chymification of the food, quotes Wm. Hunter's witty remark—"Some physiologists will have it that the stomach is a mill, others that it is a fermenting vat, others again that it is a stew pan; but in my view of the matter, it is neither a mill nor a fermenting vat, nor a stew pan; but a stomach, gentlemen, a stomach."

For a critical account of Beaumont's work, just what light he was able to shed and what he left in obscurity, reference may be made to a 32-page review and critique of Beaumont's book by D. Francis Condie of Philadelphia, published the next year (1834) after its appearance, in the 14th volume of the American Journal of the Medical Sciences. Condie quotes from Raynier who published a work "*De Digestione in Ventriculo*" in 1792. "To arrive at anything like positive conclusions in regard to this subject, the experimenter must be enabled to inspect the interior of the healthy stomach whilst its functions are going on, and study there the modifications which the composition of the alimentary bolus undergoes from its entrance through the cardia until its final escape through the pylorus—we shall then, but I fear not before, be able to say what is the nature of digestion, and what are the powers by which it is effected." Condie continues, "the opportunity here required, the occurrence of which was no doubt thought impossible by the writer just quoted, has actually beeen furnished to Dr. Beaumont. By a surgical case nearly unique in its results, the interior of the stomach in a state of health, and in the perfect performance of its functions, has been laid open to his view, and he has been able to study daily for a series of years, the actions of that important organ—to mark the successive changes produced in the food during the process of digestion and to determine with accuracy the composition and properties of the gastric fluids, and their effects upon the different kinds of aliment in ordinary use · · · · The report of his experiments and observations constitutes unquestionably, in many particulars, the most important work ever published on the physiology of digestion." Condie evidently was familiar with the scientific literature of the time, and did not hesitate to point out wherein Beaumont's observations were incomplete and he regrets that the peculiar advantages possessed by Dr. Beaumont for studying the process of digestion did not fall to the lot of some one better trained than he, particularly in chemistry. The two most important problems; in what does chymifica-

tion consist? and in what manner is it effected? Condie thinks Beaumont left still pretty much in obscurity.

That the gastric juice really has a solvent power over the most insoluble material, however, Beaumont proved in the most conclusive manner. He determined the period of time required for pure gastric juice drawn from the stomach to reduce different alimentary substances to a paste, when kept at 100°F outside the body. It is from Beaumont that we derive our school book teachings regarding the relation of fiber and toughness in food to digestibility. The articles requiring longest time for chymification were found to contain much fatty or oily material and his statement that a slight admixture of bile aids digestiblity of fatty foods to some extent, has a very modern sound.

Beaumont apparently was considerably mystified by the movements of the stomach. He is quite clear, however, on three points; one, that by alternate contraction and relaxation of the transverse muscular fibers a peristaltic motion is produced, which commences soon after the food is received, and causes the latter to "revolve around the interior of the gastric cavity." The second is that old and new food in the stomach are mixed thoroughly by a sort of "churning" of the contents. And third, that from the very beginning of chymification—from the time food is received into the stomach, until that organ becomes empty—*portions of chyme are constantly passing into the duodenum.* It is the last of these statements which forms the topic of the present discussion.

As might have been expected, many of Beaumont's observations have been wholly confirmed by later and more critically controlled observations, some have been only partially confirmed, and some few have been refuted. Carlson (1916) had an opportunity of observing a man with a stomach fistula for a period of nearly four years. This case has been called "a second Alexis St. Martin." But there is this important difference. St. Martin could chew and swallow his food directly into the stomach. Carlsons' Mr. F. V. had made the tragic mistake while a boy of trying to swallow strong caustic alkali and as a consequence the oesophagus was completely sealed. The fistula was therefore a deliberately located and circumscribed affair instead of a gaping aperature accidentally caused and crudely, though adequately, reduced surgically, as in St. Martin's case.

Carlson says of the movements of the stomach, as seen by direct observation in F. V., "the picture revealed by the gastric cavity when the empty stomach is in a period of rhythmic contractions is interesting, but rather bewildering, and we have ceased to wonder how Beaumont complete-

ly failed to grasp the character of the stomach movements in digestion, as he relied mainly on direct inspection of the stomach of St. Martin."

Carlson's method, in addition to direct inspection, was to record the motions of the stomach by means of a thin-walled, rubber balloon introduced into the stomach and connected by air transmission to a recording device. He was interested chiefly in the motions of the empty stomach or the hunger contractions which had been re-identified as the cause of the sensations of hunger by Cannon and Washburn in 1912. Records obtained from Mr. V. show the effects of a sudden dilatation of the balloon by increased pressure. The distension caused a few strong contractions which in turn caused the hunger sensations. These were felt and recorded by the subject himself. This is the behavior of the wholly empty stomach and Carlson and his pupils have shown that these strong contractions of the empty viscera gradually emerge from a series of tonus waves which begin ordinarily within 20 to 30 minutes after eating. The exact time depends to some extent on the degree of filling and to some extent upon individual idiosyncrasy. In addition to these types of movement we have of course the so-called digestion peristalses which had been seen by Beaumont and which are easily demonstrated by the X-ray and the opaque meal.

Cannon who introduced (1898) the use of the Roentgen ray for study of the gastro-intestinal movements and first demonstrated the peristaltic character of the movements of the filled stomach, demonstrated also in the cat how sensitive these movements are to strong psychic states like fear and anger. Rogers and Hardt (1915) in Carlson's laboratory found that the hunger contractions of the empty stomach both of animals and man are more easily inhibited by chemical agents introduced into the stomach and acting on the mucosa than are movements of the same type in the filled stomach. The mucosa as a matter of fact never does, in the experience of Carlson's laboratory, induce a reaction of greater activity. Anything which can act through the mucosa alone always produces inhibition. The nervous mechanism by which this is accomplished is either the short-fibered intrinsic plexus, or a center in the medulla and the splanchnic nerve.

In this connection the action of alcohol is of interest. Most people who have had the experience often enough to recognize it, claim that a glass of beer or wine or a cocktail before a meal increases appetite and possibly the hunger sensation. We must bear in mind the difference between appetite, which is simply the psychic appreciation of food depending upon the general bodily condition, and even upon such extraneous matters as the behavior of the stock market, and hunger which is the local sensation caused

by contraction (cramp) of the stomach. Carlson himself, writing in 1916, confessed that a glass of beer at meal time seemed to "awaken or increase appetite" and Pavloff has recorded an instance in his own experience where a glass of wine seemed to initiate the sensation of hunger the very minute the wine reached the stomach. Carlson therefore, from his own and from wide inquiries amongst his friends, expected to find in Mr. V. and in himself and others who submitted to the balloon method of study, that the introduction of alcoholic drinks would increase gastric tonus and initiate the contractions which give rise to hunger. To his surprise, the effect was just the opposite. Wine, beer, brandy, pure alcohol (diluted) introduced directly into the stomach always inhibited hunger contractions. If there is ever augmentation of appetite it must be through general effects on the nervous system rather than through contractions of the stomach.

Cannon's (1898) demonstration for the cat that the peristaltic wave, starting well up on the fundus of the stomach runs all the way to the pylorus, without interruption, was speedily confirmed by Roux and Balthazard (1898) on frogs, dogs, and human subjects, these authors also working with the X-ray. Greatly improved technique has substantiated this fact for the human stomach many times. Indeed, it is an every day observation now in every roentgenological laboratory. Opinion has fluctuated extensively, however, regarding two points in connection with the emptying of the stomach, one, the significance of the antrum and two, the control of the pylorus. Many roentgenologists have maintained, as did Hofmeister and Schütz (1885) from direct observation on the dog, and indeed, as Beaumont himself seems to imply quite clearly, that at times the transverse band completely pinches off the antrum, dividing the stomach into two cavities. According to this view the function of the whole pyloric portion is mainly that of discharging the contents into the duodenum. If, however, the transverse band does not pinch off completely, the pyloric portion has a triturating and mixing function was well as that of expulsion. Motion pictures of the dog's stomach exposed in a bath of salt solution, according to Alvarez and Zimmermann (1928), often clearly exhibit a systolic narrowing of the entire antrum. These authors do not find much evidence of a sphincter antri, or transverse band, but at times, the waves of contraction in the region of the incisura are deep enough to divide the stomach momentarily into two pouches. We shall encounter evidence presently that contractions of the antrum, even if serving a triturating function, are by no means random or ungoverned motions. Meantime let us see how the pylorus operates.

The whole conception of the stomach as a reservior and feeder for

the intestine depends upon the behavior of the gateway. What governs the pylorus? Richerand ascribed to this mechanism something like intelligence when he said, "it has a peculiar tact which enables it to select from the contents of the stomach what is proper to pass through, while it holds back the remainder." With less teleology but more science, Hirsch (1893) and Serdjukow (1899), a pupil of Pavloff, both obtained evidence that an acid reaction in the duodenum retarded evacuation of the stomach. Tobler (1905) a little later found that a balloon put into the duodenum and inflated kept the pyloric sphincter contracted. Marbaix (1898) speaks of intestinal repletion as the governing factor. When the intestine has had enough it signals to the sphincter to shut off the supply. However, there is another factor involved; namely, the pressure exerted by the antrum on the stomach side. The roentgenological evidence is complete that peristaltic waves descend over the stomach continuously so long as there is food in the organ. Cannon (1898) thought his evidence was conclusively clear also that the pylorus does not open for every peristaltic wave passing over the antrum. When it does open, contents are discharged, but if the sphincter holds tight, the contractions of the antrum merely churn up the contents or squeeze some of it back into the fundus. Cannon (1904) had observed very different rates of evacuation of the stomach contents, depending on the kind of food ingested by his experimental animals. Carbohydrate meals emptied most rapidly, protein meals next and fatty meals most slowly. The behavior of the pylorus must in some way be related to the character of the food. The observations of Hirsch and of Serdjukow suggested to Cannon that the free acid of the stomach contents may not only close the pylorus when acting from the duodenal side of the sphincter, but may also give the signal for relaxation of the sphincter when acting from the stomach side, and thus Cannon built up his theory of the acid control of the pylorus. Carbohydrate does not bind up the acid of the gastric juice as does protein and thus we have an explanation of the more rapid passage of the former. The delay of a fatty meal can be made to fit into this theory, for fat retards the secretion of gastric juice and causes regurgitation of bile and pancreatic juice into the stomach, as Beaumont observed on St. Martin, thus neutralizing the acidity. Cannon marshalled many facts in support of this theory, but unfortunately there were then some facts and now there are many more for which it does not afford a satisfactory explanation (See *e.g.*, McClendon, 1915).

Cannon's complete theory was published in November 1907. It was not seriously challenged for about nine years. Spencer, Meyer, Rehfuss and Hawk reported in February 1916 that with the retention tube method they

could not obtain evidence that free HCl was necessary for opening of the pylorus, and Morse (1916) in October of the same year gave detailed measurements of discharge of water and acid of varying concentration from the stomach of dogs under ether, with the dorsal spinal cord destroyed so as to eliminate splanchnic inhibition. He found that neutral water left the stomach at a more rapid rate than any concentration of acid noted. In December 1916 Cole emphatically denied the whole conception, basing his contention on exhaustive roentgenological studies of the human subject with particular reference to the duodenal cap, which Cole believes should be regarded really as a part of the stomach. He sums up by saying "there is no roentgenological evidence in man of a periodic opening and closing of the pyloric valve independently of the gastric cycle, as described by Cannon." Cole's statement, that a meal composed of mixed foods begins to be evacuated often before it is completely eaten, has been many times confirmed. Water of neutral reaction has been observed through fistulae in dogs to pass in rhythmical spurts into the duodenum (Ivy, 1918) and a meal of bismuth and milk begins to pass the pylorus of the infant's stomach within five minutes of its ingestion (Pisek and LeWald, 1913). The acid control theory would offer poor comfort for the person with achlorhydria. If the gateway never opened except under the stimulus of free acid, there are a good many people living who would never get their food to the intestine. Ortner (1927) studied the evacuation rate in dogs having a Dastre cannula in the duodenum a few centimeters below the pylorus. He found that alkaline water was passed through sooner after ingestion than acid, and that strong acid (above 0.3 per cent HCl) distinctly inhibited the relaxation of the pyloric sphincter. Luckhardt, Phillips and Carlson (1919) prepared a dog so that chyme could be collected from the duodenum and also so that the movements of the antrum could be recorded by means of the balloon method. They found material issuing from the stomach with each tonus wave of the stomach wall. This was confirmed on a human subject also by recording the movements of the stomach, after a barium meal, by the balloon method and watching with the fluoroscope for the appearance of the duodenal cap. The cap appeared always coincidentally with the record of tonus rhythm which by fluoroscopic examination consisted of a plain succession of peristaltic waves.

An equally convincing study has been made by Wheelon and Thomas. Joseph and Meltzer (1911) had observed that with each contraction of the pyloric part of the stomach, the duodenum stopped its rhythmic activity and lost its tone, only to resume it again when the contractions passed off. Wheelon and Thomas (1922) in Joseph's department at St. Louis made a

more detailed study of this phenomenon. They invented an instrument, which they call an enterograph, consisting of three small rubber-covered chambers. It is introduced through a stomach fistula of a dog and so placed that one chamber lies in the duodenum, one in the pyloric sphincter and one in the antrum. Records are taken by air transmission through thick-walled small rubber tubes which likewise issue through the fistula. By this method they made numerous records which show clearly a well-defined coordination of events in antrum, sphincter and duodenum. When the antrum is relaxed the sphincter is always closed, and the duodenum is at least partially contracted. When the antrum is contracted the sphincter is relaxed and the duodenum is at least partly relaxed. This goes on with perfect regularity for hours. "The parts excited cranialward transmit caudalward to excite lower segments," and therefore the behavior of the three mechanisms falls into agreement with Bayliss and Starling's "law of the intestine," *i.e.*, a progressive band of constriction preceded by inhibition and followed by relaxation.

It would appear from these results of Wheelon and Thomas that free acidity can scarcely furnish the stimulus for opening of the pylorus. But they did not make direct observations on the chemical changes along with their study of the motor mechanism. This deficiency has just recently been supplied by James McCann working in the department of experimental surgery at the Mayo Foundation laboratories. McCann (1929a) was interested first in the question of the control of the acidity of the stomach. Boldyreff in 1907 proposed the theory of "the self-regulation of the acidity of the gastric juice" by regurgitation of alkaline duodenal fluids into the stomach. Among other facts he showed that when 200 cc. of 0.5 per cent HCl were introduced into the stomach of a dog, the acid was neutralized by regurgitation of duodenal alkalies, chiefly in the pancreatic juice. The rise of trypsin concentration in the stomach contents during the latter part of the fractional test meal by the retention-tube method of Einhorn and of Rehfuss has been held by some clinicians to be in accord with Boldyreff's theory. McCann tested this hypothesis by eliminating the duodenal juices by draining them directly into the ileum. In other experiments he resected the antrum of the stomach entirely, thereby eliminating the alkaline mucous secretion of the antrum, to see whether it might play any part in the control of acidity. Seven dogs were subjected to the procedure of duodenal drainage, but fractional analyses of the stomach contents after this operation, and feeding with meat or stimulating gastric secretion with histamine, did not show any variations from the curves found before operation. Addition of carbohydrate and fat to the protein meal showed the same altera-

tions of the curves obtained by fractional analyses in operated dogs as in normal dogs. Regurgitation of alkali therefore is not an essential mechanism in the control of acidity. He concludes that the mechanism is essentially an intragastric one, and that the relationship between the concentration of free HCl and the rate of secretion agree with Pavloff's observations on gastric pouches. "It is a rule almost without exception," says Pavloff, "that the acidity of the juice is closely dependent on the rate of secretion; the more rapid the latter the more acid the juice, and *vice versa*." In McCann's dogs with the antrum resected there was increase in the rate of secretion or in the concentration of acid, such as is seen in normal dogs, indicating that the prepyloric segment is in some way concerned in the regulation of secretion.

In his second study McCann (1929b) correlated his method of fractional analysis of the gastric secretion with roentgenological studies of the motor phenomena. The curves of free acid, total acid and neutral chloride were plotted and roentgenograms taken at certain definite points on these curves. They show that the stomach begins to empty very soon after the meal is ingested and the rate of emptying increases progressively from early digestion until the process is complete, quite independently of free acid in the contents. "The emptying depends not on acidity but on progressive relaxation of the whole pyloric end of the stomach. The activity of this *pars pylorica*, of which the sphincter is merely the most efficient segment, depends on its irritability and, in these studies, on the stimulating action of raw protein to tonic and peristaltic action. With the disintegration of the protein to the products of digestion, there is a graded induction in the intensity of the stimulus to the antrum and consequently a progressive relaxation which permits more rapid evacuation."

Katsch (1927) of Frankfort has pointed out that we cannot consider the motor and secretory functions of the stomach as independent. Katsch's statement of stomach function in a single sentence is this, "to receive the food, hold it for a time, change it chemically and physically and, in proper rhythm with these changes, to pass the food along to the intestine." This conception, obtained from the clinic, is identical with that of McCann obtained from the experimental laboratory. Many of the gastro-intestinal disturbances have their origin in a breakdown of this nice coordination of movement to secretion and digestion. Too rapid evacuation may have identically the same consequences as too little secretion. It must be remembered too that the motility of the stomach has the purpose not merely to deliver the food to the intestine where the bulk of digestion takes place, but also to remove it from the stomach as rapidly as digestion takes place,

in order that digestion may further proceed. The accumulation of digestive products slows digestion just as the product of any mill slows up the mill unless removed promptly. The intestine gets rid of its products of digestion by absorption, but absorption does not take place in the stomach. The only means of getting rid of its products, partially digested only, as a rule, is to pass them along. Thirty years ago we thought of the churning, mixing motions as characteristic of the stomach and peristalsis as characteristic of the intestine. We must now reverse the order; peristalsis with regular rhythmic emptying is more characteristic of the stomach, while mixing, accomplished by rhythmic segmentation and peristalsis is more characteristic of the intestine. These conceptions were established mainly by X-ray studies on animals and on the human subject and the operative experimental work on animals has only recently caught up with it. The one method has the advantage of observing the relatively undisturbed organ; the other the advantage of direct approach by operation and direct chemical examination of contents. Both methods are essential.

Rehfuss and his collaborators (1919, 1920), particularly Hawk and Bergeim, using the retention tube method, have added much information to what we knew before regarding the digestibility of—or, as they prefer to call it, the "gastric response" to—different foods. In the course of this work they observed that stomachs differ greatly in their response to the same kind of food. Some respond promptly and decidedly to the entrance of food, whereas others respond slowly and indifferently. One type of stomach empties very quickly whereas a second type evacuates slowly under like dietary conditions. They grouped their subjects into the rapid-emptying and slow-emptying types and found, for example, with test meals of 100 grams of beef the average emptying time for the former was 2 hours and 35 minutes, for the latter 3 hours and 25 minutes. They do not make a particular point of it, but doubtless Rehfuss and Hawk would agree, that there are all sorts of gradations in these two classes, so that the real contribution which they made ten years ago is, that stomach-emptying time,* like reaction time to sound, or any one of a dozen other physiological reactions which can be measured in time, is decidedly an *individual* matter. The rather extensive variability in the type of peristalsis seen in different animals, or even in the same animal at different times (Alvarez, 1928), probably affords sufficient basis for this individual gastric behavior.

Some clinics, as, for, example, that of Katsch at Frankfort, have now adopted instead of a test meal, some standard stimulant to the gastric

* Mattill and Smith (1930) on another page of this number show curves of average evacuation rate in rats fed on different cereals.

glands like 20 per cent alcohol or pure caffeine. Katsch uses also a standard color dissolved in the stimulating solution. Two drops of 2 per cent methylene blue in 300 cc. of water containing two tenths of a gram of *caffeinum purum* he finds sufficient. For one half hour after introduction of the retention tube, he draws small samples every ten minutes in order to establish a base line of free acidity, total acidity, total chlorides and pepsin. Then the stimulating solution is introduced and with the tube still in place he draws a 10 cc. sample every ten minutes, compares the color with the original color of the solution and again determines in each sample the acidities, chlorides and pepsin. The color chosen enables him to detect any bile regurgitated. Katsch rightly objects to calling the resulting curves which are obtained in this way "secretion curves." They should be called acidity curves, chloride curves and pepsin curves; for even with pure solutions as stimuli what one draws up is never pure gastric juice, except after the test solution is completely removed, and even then one may find bile, pancreatic juice or saliva. To give a couple of examples only. There is a certain type of motor-compensated hypersecretion in which the stomach is excited to produce a great deal of juice; but a hypermotility removes it rapidly, so that only a small amount of content is found. Again a late-acid curve may be given in certain cases of gastritis where the secretory power is weak and in cases of partial stenosis of the pylorus the curve of acidity may be identical.

How then is the function of secretion related to the motility of the stomach? It will be necessary to state rather dogmatically the results of recent work. Pavloff's demonstration thirty years ago of the psychic secretion of gastric juice through the vagus and the demonstration by Babkin, one of Pavloff's most brilliant students, that the secretion of the stomach, like that of the salivary glands, may be educated to respond to a conditioned reflex, has brought the secretion of this organ, like its motility, definitely into the psychological realm. No longer can we doubt that both motor and secretory activities of the stomach are subject to a certain degree of control from the higher centers, for both motility and secretory phenomena, especially the conditioned reflex type of secretion, are affected by obliteration of the cerebral cortex in dogs. This central or reflex control of secretion, however, affects ordinarily only the first gush of gastric juice which occurs before the meal is taken or while it is being appreciated through the gustatory nerves. What regulates the flow of gastric juice after the meal is safely in the stomach? Following the demonstration by Bayliss and Starling of secretin as the hormone of the pancreas providing for the flow of that juice as long as acid chyme is coming into

the intestine, Edkins (1906) first demonstrated the presence of a similar hormone in the stomach, later named *gastrin* by Keeton. Ivy and Farrell (1925) have now placed the hormone control of the stomach beyond the shadow of a doubt by their transplantation experiment. Cutting off a little bag of stomach wall in the fundus with its blood supply, Ivy transplanted it into the mammary gland of a dog which had recently suckled a litter of pups. Waiting until a new blood supply was established from the mammary vessels to this little stomach, he then severed the original supply including any extrinsic nerves. A fistulous opening into the pouch enabled him to study the rate of secretion from its gastric glands. Whenever the dog was fed, the pouch secreted. The only possible connection between the stomach remaining in its normal position and the transplanted pouch was by way of the blood supply. The substance which stimulates (*i.e.*, gastrin) is formed whenever food reaches the pyloric portion of the stomach. It is formed by the gastric mucosa in that region, absorbed into the blood, and carried normally around through the general circulation and back to the fundic glands. In Ivy's experiment it is not certain that gastrin was the agent but something in the blood was carried also to the transplanted pouch and caused it to secrete. Various substances in the pyloric region, like extract of meat, products of protein digestion, soaps, lactic acid, butyric acid, pancreatic juice, bile, soda solution, dextrose, extracts of vegetables and alcohol, can stimulate the production of gastrin and thus indirectly stimulate the fundic glands where only HCl as well as pepsin is formed. Moreover, these same substances stimulate the production of gastrin, not so effectively, but demonstrably, when introduced into the duodenum (see Keeton and Koch, 1915). We thus have a provision for the continuous production of gastric juice after we have lost interest in the food from the psychological point of view.

The secretion of gastric juice therefore would seem to be related to the emptying movements of the stomach by having a common cause. McCann's recent work indicates, if it does not prove, that the emptying depends on irritability of the pyloric region and on the stimulating action of the food to produce tonic and peristaltic contraction. Food in this same region excites the production of gastrin and consequently of gastric juice. Demonstration of a direct relationship between the two phenomena is approached in the experiments of Ivy and Farrell (1926) in which the transplanted gastric pouch was shown not only to secrete when the stomach proper secreted, but also to contract when the stomach proper contracted and to rest when it rested. Does the common cause act on the two mechanisms by means of the same agency or only simultaneously? Babkin

(1928) has reported recently on some Russian work done in 1923 and 1924 which shows that extract of beef put into the circulation can stimulate intestinal movements and when placed in an isolated loop of intestine can cause movements in another loop with which the first is connected only by way of the blood. Is there a motor hormone produced in the pyloric region of the stomach by absorption of food or is the food itself, after absorption, the stimulating agency to produce relaxation of the pyloric musculature and increased tonus and peristalsis above?

Whatever may be the explanation, it is quite clear that modern work tends to confirm the statement of Beaumont that "from the time food is received into the stomach until that organ becomes empty, portions of chyme are constantly* passing into the duodenum, through the pylorus." This takes place slowly at first, "but is rapidly accelerated towards the conclusion of digestion, when the whole mass becomes more or less chymified." "As the food becomes more and more changed from its crude to its chymified state, the acidity of the gastric fluids is considerably increased . . . . and the general contractile force of the muscles of the stomach is augmented . . . . giving the contained fluids an impulse toward the pylorus" (loc. cit. 113).

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* *i.e.*, as judged by a steady decrease in volume of stomach contents—Ed.

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